Soil microbial diversity and ecosystem functioning in smallholder African agroecosystems

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Submitted in partial fulfillment of the

requirements for the degree of

Doctor of Philosophy

in the Graduate School of Arts and Sciences

COLUMBIA UNIVERSITY

2015

ABSTRACT

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In this dissertation I assess the effect of fertilizer-based efforts to increase crop yields on smallholder African farms (the African Green Revolution) on the diversity and functional capacity of soil microbial communities and the ecosystem processes they regulate. In the introduction I provide a brief overview of the African Green Revolution and its critiques. In chapter 1, I advocate for the application of a functional trait-based approach to agroecology. I propose a functional trait-based approach to understanding the contribution of biodiversity to ecosystem services in agriculture. In chapter 2, I assess the impact of organic and mineral fertilization on the taxonomic composition and functional capacity of soil microbial communities in western Kenya. In chapter 3, I attempt to link these patterns in taxonomic and functional capacity to ecosystem process rates, specifically denitrification potential and carbon mineralization. Finally, in chapter 4, I measure fast- and slow-cycling organic matter fractions and their relationship to crop production and to the microbial enzymes that drive their turnover. Common to all chapters is the theme that short- and medium-term efforts to improve agricultural production through nutrient addition may feedback on the processes that sustain agriculture. This is in contrast with most research on the impacts of agricultural intensification, which tend to assess environmental impacts *per se* such as eutrophication and greenhouse gas emissions. I provide a summary and recommendations for future research in the conclusions.

TABLE OF CONTENTS

List of Tables, Figures, and Boxes **iii**

Acknowledgements **v**

Introduction **1**

Chapter 1. Functional traits in agriculture: agrobiodiversity and ecosystem services **6**

1.1 Abstract *6*
1.2 The utility of a functional trait approach in ecology *6*
1.3 Proposed trait-based approach to agriculture *10*
1.3 Applications of a trait-based approach to agriculture *14*
1.5 Using traits to generate agroecosystem management strategies *17*
1.6 Concluding Remarks *19*
1.7 Glossary, Boxes, and Figures *20*

Chapter 2. Agricultural intensification and the functional capacity of soil microbes on smallholder African farms **31**

2.1 Abstract *31*
2.2 Introduction *32*
2.3 Materials and Methods *34*
2.4 Results *41*
2.5 Discussion *44*
2.6 Tables and Figures *48*

Chapter 3: Farm management, not soil microbial diversity, controls nutrient loss from smallholder tropical agriculture **60**

3.1 Abstract *60*
3.2 Introduction *61*
3.3 Materials and Methods *63*
3.4 Results *71*
3.5 Discussion *72*
3.6 Tables and Figures *76*

Chapter 4: Opposing effects of different soil organic matter fractions on crop yields **83**

4.1 Abstract *83*
4.2 Introduction *84*
4.3 Materials and Methods *87*
4.4 Results *95*
4.5 Discussion *99*
4.6 Conclusion *103*
4.7 Tables and Figures *104*

Conclusion **117**

The importance of vegetation *117*
Microbial functional capacity *118*

Soil organic matter changes and effects *119*

Bibliography **121**

LIST OF TABLES, FIGURES, AND BOXES

Chapter 1

Glossary 20

Box 1. Trait-based framework for agroecology 23

Box 2. Outstanding research questions 26

Figure 1. A trait-based modeling approach to creating management targets. 29

Figure 2. Potential trajectories of functional trait space in agriculture. 30

Chapter 2

Table 1. Farm selection criteria 48

Table 2. Drivers of microbial relative abundance on experimental plots 49

Table 3. Drivers of microbial relative abundance on actively managed farms 50

Table 4. Soil properties and crop yield 51

Table 5. Regression model results of taxonomic diversity 52

Table 6. Regression model results of functional gene abundances 53

Table 7. Model results for abundances of additional functional genes 54

Figure 1. Map of the study area 55

Figure 2. Taxonomic diversity response to fertilization 56

Figure 3. Community composition response to fertilization 57

Figure 4. Coefficient of variation increases with fertilization 58

Figure 5. Catabolic multifunctionality is highest under legume rotation 59

Chapter 3

Table 1. Means and standard deviation of key variables 76

Table 2. Model result statistics 77

Figure 1. Image and map of the study area 78

Figure 2. Path diagrams for structural equation models 79
Figure 3. Community composition response to taxonomic diversity 80
Figure 4. Functional diversity and gene abundances 81

Chapter 4

Table 1. Soil properties, organic matter fractions, and enzyme stoichiometry by treatment 103
Table 2. Nonlinear model of crop yield response to organic matter fractions 104
Table 3. Parameter estimates and model statistics for structural equation model 105
Table 4. Mean isotope values for different components of the maize plant 106
Table 5. Enzymes studied, the most relevant nutrients, and their particular function 107
Table 6. Activities of individual enzymes included in assay 108
Table 7. Regression results for organic matter and microbial enzyme models 109
Table 8. Regression results for other soil organic matter properties 110
Figure 1. Coefficient plots for SOM 111
Figure 2. Yield response to particulate and mineral-associated organic matter 112
Figure 3. Coefficient plot for enzymes 113
Figure 4. Structural equation model for 2013 114
Figure 5. Structural equation model for 2012 115

ACKNOWLEDGEMENTS

Proper thanks isn't given through lists and descriptions. Acknowledgements are lived; they're phone calls, dinners shared, or a hug after a long time apart. The number of people who have supported me in this process is greater than I imagined at the outset. If I've been able to communicate to all of you how much your support has meant to me, then that is enough of an accomplishment to have made this process worthwhile. I hope that the acknowledgements below will be a reminder of my gratitude.

First, I thank my two supervisors, Shahid Naeem and Cheryl Palm, for their support. Despite all of their commitments, they found time to discuss ideas, write letters of support, and provide detailed feedback on written work. I have benefitted tremendously from their depth of knowledge and acute insight. Their feedback helped me shape nascent interests into a tangible project and I have grown greatly in my capacity to think critically, ask questions, and write. Special thanks also to my 'unofficial' supervisor, Mark Bradford, for orienting my look belowground while I was a Masters student. My background was in philosophy and economics and, for better or worse, Mark turned me into an ecologist. Mark gave me more attention, direction, and feedback than most students get from their primary supervisor. I also learned a tremendous amount from Mark's lab, especially Ashley Keiser, Robert Warren, and Mike Strickland. My other committee members—Krista McGuire and Justin Wright—have also been a great support by combing through drafts, spending hours in committee meetings and in one-on-one discussion. Krista gave me access to her lab and taught me how to do DNA extractions, without which much of this work wouldn't have happened.

Several people have been essential to the carrying out of this project, from fieldwork to manuscripts. Wilson Ondiala and Steve Ogendo tirelessly assisted with all aspects of fieldwork. Herine Okoth was indispensible in the field, identifying and interviewing farmers. Anna Wade helped both in the field and the lab and Madeleine Rubenstein and Jeff Smith helped with lab work. Jack Gilbert, Matt Wallenstein, and Joe Zhou have been great co-authors and committed resources for the microbiological analyses that are core to this dissertation. Kate Tully was indispensible to all phases of this work and was a great friend as well as a collaborator. Thanks to all other co-authors for their intellectual collaboration and feedback: Maya Almaraz, Colin Bell, Fabrice DeClerck, Danny Karp, Claire Kremen, Chris Neill, and Noah Sokol.

The Department of Ecology, Evolution, and Environmental Biology at Columbia was a great home during my dissertation and Maria Estrada-Werst, Lourdes Gautier, Amy Kohn, and Jae McFadden were the foundation. Many thanks to E3B faculty for teaching great classes, shaping ideas, and asking tough questions – especially Joel Cracraft, Ruth DeFries, Duncan Menge, and María Uriarte. The community of students and postdocs made for a great working environment and I'm especially appreciative of the conversations, lab meetings, and WEEDS sessions with Megan Cattau, Sarah Guindre-Parker, Meha Jain, Jesse Lasky, Liz Nichols, Elsa Ordway, Case Prager, Naomi Schwartz, Ben Taylor, Brian Weeks, and Rae Wynn-Grant.

The Agriculture and Food Security Center at the Earth Institute was my home-away-from-E3B. Phil Fitzpatrick, Andrew Miller, Mary Pasquince, Allison Rose, and Janelle Sommerville provided fantastic administrative and technical support. I also benefitted immeasurably from the many scientists affiliated with the AgCenter; I'm especially grateful for the interactions I've had with Pedro Sanchez, Jonathan Hickman, and Hervé Bisseleua. This dissertation was supported by several grants, including a Lewis and Clark Fellowship from the

American Philosophical Association, a Leitner Fellowship from the Institute of African Studies at Columbia University, a Fulbright Fellowship, and a Borlaug Food Security Fellowship.

I was able to complete this project because of a life away from work with my close friends in Brooklyn. Special thanks to my roommates Chad and Boubs. Climbing with Chad and Elsa (and Shane when I made it to New Haven) was a much-needed forum for decompression. And of course, as always, Dave and Fish for being my best pals. Though I was always a student at Columbia University, the writing of this dissertation largely happened in Montpellier, France; Madison, Wisconsin; and Dakar, Senegal. These periods of intellectual incubation would not have been possible without the wonderful hosts I had. In Montpellier, Fabrice DeClerck gave me space to work at Bioversity International. Harold Duruflé helped keep me from being too productive with regular coffee breaks and political discussions. Nicolas Mouquet and Tanguy Daufresne were monster rock climbing partners, intellectual collaborators, and Nico a grade-A+ microbrewer. In Madison, Randy Jackson and Monica Turner welcomed me into their lab groups and Gary Oates connected me with working space while always had an extra minute to chat about baseball. Special thanks to Garrett Nelson for afternoons sailing rather than working and New England nostalgia; Kramer Gillen, Becca Summer, Rachel Boothby, and everyone else for camping, canoing, brunches, etc.; Scott Laeser and Chelsea Chandler for putting me to work on their farm; Kevin and Lauriane Garcia *pour le dépaysement au sein du Wisconsin*. In Dakar, Dominique Masse welcomed me into the IESOL lab at IRD. John Cropper, Nik Sweet, Pape Diallo, and Falaye Danfakha were great for long conversations and a beer (or soda, for some!).

Though the material in this dissertation is from Kenya, the inspiration comes from Senegal, and particularly the region of Kédougou and village of Togué. For everyone I've known

in Kédougou: *on diaraama*. Special thanks to Mamdou Aliou Diallo, Chris Hedrick, and Famara Massaly for giving me the support to develop this connection to place.

And then, of course, there's family. Thanks to my host family in Thiès and everyone in Togué for showing me that family is a much broader concept than what I grew up with. Thanks to Andy Tanner, Sam Kesner, and my *jaaja,* Robyn d'Avignon, for years of stimulating discussions and personal support. Erin Kitchell has been a partner in this whole process *sans égale*. She's been a roommate, friend, traveling companion, sounding board, and source of advice. For my parents Jon and Maryann and my sister Kate, what comes to mind is that the dissertation wouldn't have happened without their support, in all of its forms. But I think that misses the point: without family I wouldn't be who I am, and that's much more meaningful than the completion of a degree.

To Manga Camara

Ko kanɓe hoolii lan laawol ngol. O thèse on ko teddungal maɓɓe.

INTRODUCTION

Exponential growth in global crop production in the post-World War II era—the Green Revolution—is so well known that it is almost trivial to point out (e.g. Evenson and Gollin 2003, Hazell and Wood 2008). Such drastic transformation of agronomic possibility due to crop breeding and fertilizer manufacturing has facilitated equally rapid transformation in human diets away from plant consumption and towards meat (Bonhommeau et al. 2013). These changes in dietary demands have increased the amount of land needed for food production (Kastner et al. 2012), creating pressure on the environment.

A common caveat to these broad patterns is that they are strongly geographically heterogeneous (e.g. Hazell and Wood 2008, Kastner et al. 2012, Bonhommeau et al. 2013). While global crop production has increased precipitously, it has remained stagnant—and even fallen, per capita—in sub-Saharan Africa (Hazell and Wood 2008), with modern varieties contributing less to yields in sub-Saharan Africa than elsewhere (Evenson and Gollin 2003). Diets in sub-Saharan Africa have not followed global trends and remain based on staple grains (Bonhommeau et al. 2013) while depending on access to non-cultivated foods, such as bush meat, to meet nutritional needs (Golden et al. 2011).

Continued low production and productivity in sub-Saharan Africa has motivated the international development community to invest in a new green revolution for Africa (Toennissen et al. 2008). This African Green Revolution aims to increase crop yields through simultaneous investment in high-yielding crop varieties, mineral fertilizers, building soil quality, and applying recommended agronomic practices (Toennissen et al. 2008, Sanchez et al. 2009). In Malawi, there has been strong investment in fertilizer subsidy programs aimed to increase farmer uptake

as well as increased use of improved crop varieties (Denning et al. 2009). This approach has been extended at a larger scale by the Millennium Villages Project, which aims to increase use of similar techniques at 14 sites across sub-Saharan Africa (Sanchez et al. 2007, 2009). Initial results suggest that these approaches have increased yields among participating farmers (Sanchez et al. 2009) and even in national production data (Denning et al. 2009). These potential successes have prompted investment by social enterprises in Africa—such as myAgro and One Acre Fund—to help farmers save for and invest in packages of seeds and fertilizers to achieve yield targets.

Though the African Green Revolution model has clear potential for achieving production targets, it has also generated critiques and concerns. Some assert that the assumption that African agriculture is performing poorly should be more nuanced (Haggblade and Hazel 2009, Roling 2010, Pretty et al. 2011). Pretty et al. (2011) argue that though yields are stagnant, net production—production minus seed required for the next cycle—has increased. Though there is need for productivity gains, there are already many examples of investments in sustainable agriculture in the region, including crop variety improvement, integrated pest management, soil conservation, and new systems of production, like conservation agriculture and System of Rice Intensification (Pretty et al. 2011). There are also, however, concerns that the estimated benefits of these approaches have been exaggerated (Krupnik and Sarr 2008, Glover 2011, Palm et al. 2014, Pittelkow et al. 2015). Relatedly, calls for increased productivity in sub-Saharan Africa often assume that low-fertility, or even degraded, soils across the continent are a primary limiting factor for crop production (Tully et al. 2015). Some have challenged this assertion showing that, at the local level, there can be high investment in soil fertility and that the paradigm of degraded

African soils does not acknowledge "grassroots" investments in soil quality (Richards 1985, Fujiu et al. 2014, Frausin et al. 2014, Fraser et al. 2014).

Others argue that efforts to intensify agricultural production overly focus on production. For the African Green Revolution to truly achieve food security, greater emphasis needs to be placed on the distributional and qualitative aspects of food production, such as greater nutritional quality (Negin et al. 2009), more equal distribution of opportunities (Loos et al. 2014)—for example, between genders (Negin et al. 2009)—and more focus on the empowerment of individuals and communities to decide how their needs are met (Loos et al. 2014). Failure to address these issues can isolate communities from the development process and be a major blockage to achieving goals for economic development, environmental protection, or "sustainability" more broadly (Cash et al. 2003, van Kerkhoff and Lebel 2006).

To address this, agricultural development should focus less on applying a one-size fits all solution and more on developing techniques that meet the needs of particular farmers in particular places (Richards 2010, Horlings and Marsden 2011). Richards argues that African farmers are highly attuned to the multiple advantages and limitations of proposed technologies and intended benefits are only one of many factors influencing decision making (Richards 1985, 1989). For instance, the adoption of agroforestry techniques in western Kenya to increase soil fertility is influenced mostly determined by labor demands, immediate benefits (or lack of benefits) to crop yields, prestige, (lack of) access to credit programs, and perceived (and real) economic benefits (Kiptot et al. 2007). Technology adoption is a highly dynamic process (Kiptot et al. 2007) and to ensure that new technologies meet the needs of farmers, farmers should thus be included as collaborators in the process of producing these technologies (Richards 2010).

There is also concern about potential environmental damages from increased mineral fertilizer use associated with the African Green Revolution. Large increases in mineral fertilizer use are generally accompanied by losses of excess nutrients to the atmosphere as greenhouse gases or to water systems (e.g. Vitousek and Aber 1997, Vitousek et al. 2009). There is emerging evidence that high levels of mineral fertilizer application in sub-Saharan Africa can lead to large N_2O emissions from soils (Hickman et al. 2015). Projections suggest that N_2O emissions in sub-Saharan Africa may double by 2050 from 2000 levels (Hickman et al. 2011). It is also possible that fertilizer addition associated with the African Green Revolution will affect ecosystem processes that feedback to agriculture itself, such as soil organic matter formation and decomposition and soil nutrient availability. Nutrient addition is known to change the composition of soil microbes, which carry out these ecosystem processes (e.g. Ramirez et al. 2010, 2012). It is unknown whether these fertilizer-induced changes feedback to the functional activity of these organisms and to the processes themselves in ways that could affect agriculture.

In this dissertation I assess this particular potential consequence of the African Green Revolution: how the increased use of mineral fertilizers and fertilizers paired with organic inputs impact the diversity of soil microbial communities and their ability to carry out soil nutrient cycling processes that are key to sustainable agriculture over the long term. In **Chapter 1** I propose a strategy for assessing the impact of biodiversity on ecosystem services in agricultural settings. This strategy incorporates recent advances in ecology that apply functional trait-based research across spatially complex landscapes, trophic levels, and to generate management strategies that maximize ecosystem services. In **Chapter 2** I apply genomic and catabolic measurement of soil microbial communities to determine the consequences of mineral nutrient addition and legume rotation-based agroforestry on smallholder farms in western Kenya. Results

suggest that microbial taxonomic diversity is negatively associated with mineral fertilizer addition, but functional diversity is increased, along with crop yields, under legume rotation. This suggests important potential synergy between management strategies that increase crop yields and that build the functional capacity of soil microbes. In **Chapter 3** I connect microbial diversity to potential denitrification and carbon mineralization. I show that farm management is a stronger direct predictor of potential nutrient loss than changes in microbial diversity due to farm management. Though experimental reductions in microbial diversity have elsewhere been shown to be a key driver of denitrification, my findings suggest that realistic changes in microbial communities may be less important contributors to ecosystem process rates. Finally, in **Chapter 4** I show that crop yields are negatively related with long-term soil organic matter fractions, which challenges widely held opinion that long-term build up of soil organic matter is beneficial for food security. I find that short-term soil organic matter pools are positively related to crop production, but only under unfavorable weather conditions, suggesting that soil organic matter may play an important role in buffering crop yields against variable weather.

CHAPTER 1. FUNCTIONAL TRAITS IN AGRICULTURE: AGROBIODIVERSITY AND ECOSYSTEM SERVICES

Published as:

Wood, S.A., Karp, D.S., DeClerck, F., Kremen, C., Naeem, S., Palm, C.A. 2015. Functional traits in agriculture: agrobiodiversity and ecosystem services. Trends in Ecology and Evolution: 30, 531-539, doi:10.1016/j.tree.2015.06.013.

1.1 ABSTRACT

Recent work on functional traits has led to greater understanding of the impacts of biodiversity in ecosystems. Yet functional trait approaches have not been broadly applied to agroecosystems and understanding of the functional importance of agrobiodiversity remains limited to a narrow range of ecosystem processes and services. To improve this understanding, we argue for a functional trait approach to agroecology analogous to that in broader ecology. We propose a trait-based approach to agriculture that adopts recent advances in trait research for multi-trophic and spatially heterogeneous ecosystems. We argue that traits should be measured across environmental conditions and agricultural management regimes to help predict how ecosystem services vary with farm practices and environment. This knowledge should be used to develop management strategies that can be easily implemented by farmers to manage agricultural systems to provide multiple ecosystem services.

1.2 THE UTILITY OF A FUNCTIONAL TRAIT APPROACH IN ECOLOGY

The loss of biodiversity due to anthropogenic activity can markedly modify the functional properties of ecosystems and the services they provide (Naeem et al. 2012). Biodiversity impacts ecosystem properties and processes because species (and individuals) differ in their contributions to ecosystem functions (*sampling effect*, see Glossary), how they use resources (*resource partitioning*), and how they modify their surrounding environment in ways that impact other species (*facilitation*; the latter two mechanisms referred to as niche complementarity (Flombaum et al. 2014)). The functional characteristics of species (i.e. their traits) influence ecosystem functioning directly through changes in biotic controls (e.g. predation or competition) and indirectly through changes in local environment (e.g. micro-climates or disturbance regimes) (Chapin et al. 2000). Traits govern not only the impacts of species on the environment, but the response of species to the environment and thus their fitness (Lavorel and Garnier 2002). Functional trait diversity, rather than the diversity of species *per se*, is therefore the dimension of biodiversity most directly related to ecosystem functioning (Naeem and Wright 2003, Cadotte et al. 2011).

Variation in functional trait diversity due to land management can be a strong driver of ecosystem functioning and ecosystem services (Figure 1). Functional traits can be assessed at different levels of biological resolution from functional groups (e.g. legumes) to species-level means (e.g. average N_2-fixation rate), to, at the finest scale, intra-specific variation (e.g. individual N_2-fixation rates). The appropriate scale of analysis depends on the importance of individual variability for the ecosystem process of interest (Wright et al. 2006, Albert et al. 2010b, 2010a, Bolnick et al. 2011, Violle et al. 2012).

In agriculture, many have suggested the importance of biodiversity to ecosystem service provisioning (Giller et al. 1997, Altieri 1999, Swift 2004, Jackson et al. 2007, Hajjar et al. 2008).

We argue that a trait-based approach to agriculture that is analogous to that applied in broader ecology could help better identify the mechanisms underlying the role of agrobiodiversity in providing agroecosystem services. This knowledge is crucial for predicting how changes in environment and management practices will impact the multiple ecosystem services provided by agriculture (Zhang et al. 2007, Power 2010, Kremen and Miles 2012), from soil nutrient cycling to pest regulation.

Because functional trait diversity is more directly related to ecosystem functioning than other dimensions of biodiversity, functional trait approaches have produced greater predictive understanding of the controls on and impacts of biodiversity across scales and ecosystem processes, even when species composition differs. By measuring quantifiable traits across a range of abiotic and biotic conditions, trait-based approaches to ecology have been able to distinguish the mechanisms underlying the impact of biodiversity on particular ecosystem processes. For example, niche complementarity has been shown to be an important mechanism influencing primary production because communities with a diversity of plant traits, such as photosynthetic type, ability to fix nitrogen, and root architecture, have high primary productivity (Dimitrakopoulos and Schmid 2004, Kirwan et al. 2007, Schumacher and Roscher 2009). Rates of nitrification, by contrast, are influenced more by dominant leaf traits than by trait diversity (Laughlin 2011) and are thus controlled more by the sampling effect. Trait-based research has also illustrated that the impact of biological communities on ecosystem processes depends on trophic interactions. For instance, predator traits, such as hunting behavior, can induce shifts in the physiology and foraging activity of prey that can cascade to modify nutrient cycling processes (Schmitz 2008, Hawlena et al. 2012). Thus, trait research provides insight into the

importance of species responses to and effects on the environment as mechanisms for biodiversity-ecosystem functioning relationships (Lavorel and Garnier 2002).

Managing for multiple ecosystem functions simultaneously (ecosystem multifunctionality) has become a key goal for agroecosystem management (Renting et al. 2009). However, the effects of biodiversity on multifunctionality are often context dependent because different mechanisms govern different ecosystem processes (Bradford et al. 2014c). Managing for multiple agroecosystem services therefore requires understanding both the responses of individual services to changes in environment and management practices as well as trade-offs that exist among services (Bradford et al. 2014c, 2014b). For instance, emerging evidence suggests that there may be trade-offs in the response of soil C sequestration and crop yields to soil organic matter build-up and that these two services are driven by different underlying mechanisms (Wood et al. in review). Because the mechanistic foundation of a trait-based approach, it could be used to develop agricultural and land-use management strategies to provide multiple ecosystem services that take into account such trade-offs.

Despite the need for predictive understanding of how changes in agrobiodiversity impact agroecosystem multifunctionality, functional trait approaches remain largely under-applied to agriculture (de Bello et al. 2010). This may be in part because principles derived from trait-based research in broader ecology may not apply to agroecosystems: many ecological processes that operate in more natural conditions (e.g. dispersal, colonization, etc.) are strongly modified by human activity in agroecosystems. In addition, people directly manipulate some traits relevant to agroecosystem services (e.g. crop breeding). Therefore, there is an urgent need for a special trait-based research agenda for agriculture. To develop generalizable principles of how agrobiodiversity impacts ecosystem processes and services, we propose a trait-based approach to

agriculture that adopts recent advances in trait research for multi-trophic and spatially heterogeneous ecosystems (Box 1). We argue that traits should be measured across environmental conditions and agricultural management regimes to help predict how ecosystem services vary with agricultural practices and environment. This knowledge should then be used to develop particular trait-based management strategies that can be implemented in farming systems to increase multiple ecosystem services (Box 1).

1.3 Proposed trait-based approach to agriculture

A trait-based approach to the study of agroecosystems could transform understanding of the importance of agrobiodiversity from largely context-specific and based on species identities to generalizable and predictive. For instance, although it is currently well established that intercropping can increase crop yields through niche complementarity (Brooker et al. 2015), understanding of intercropping comes from examples of particular species interactions in particular contexts, rather than on principles that can be generally applied across different species compositions and environmental conditions. The statement that intercropping maize with cowpeas increases yield is less generalizable than the finding that under conditions where plant-available NO_3^- concentrations are lower than a certain threshold, intercropping facultative N_2-fixing species increases staple grain seed set and protein content. The latter statement refers to well-defined, measurable traits (categorical: N_2-fixation; continuous: biomass, grain protein content) while the former refers to taxonomic affiliations that group multiple traits, thereby masking the mechanisms of *how* intercropping increases yield. Both approaches predict that intercropping increases yield, but the approach referring to functional traits can guide management strategies over a broad gradient of environmental conditions by pinpointing the general controls—abiotic (e.g. soil [NO_3^-]) and biotic (e.g. nematode inhibition of symbiosis)—

on rates of soil nutrient cycling (e.g. N_2-fixation) and human nutrition (e.g. crop yield, protein content).

1.3.1 Traits across spatial scales

Spatial heterogeneity determines the functional structure of agricultural landscapes (Fahrig et al. 2011, Mitchell et al. 2014, Nowak et al. 2015). Agroecosystems range in complexity of the spatial arrangement of crop varieties, species, fields, and landscape types. For example, intensive monocultures typically contain only a small number of crops over a large area (Lin et al. 2011), while diversified agricultural systems often include multiple crops, different land use types, hedgerows, and patches of natural vegetation such as riparian corridors (Tscharntke et al. 2005, Kremen et al. 2012). Landscape heterogeneity can have important effects on ecosystem processes by determining the persistence, distribution, dispersal, and interactions of farmland biodiversity (Kremen et al. 2007, Mitchell et al. 2015). These population- and community-level processes (determined by species' response traits) can in turn affect ecosystem services through effect traits. For instance, the ecosystem services provided by agrobiodiversity in small patches may be susceptible to an ecosystem service debt in which the services provided by these species can diminish if the species are susceptible to extinction debt due to population processes in small patches (Isbell et al. 2014). Thus, adding a spatial approach to functional trait approaches in agriculture will help predict the effects of such changes in agrobiodiversity on ecosystem services across a spatially varied landscape.

A spatial, trait-based approach to agrobiodiversity requires connecting species' traits to ecosystem functions and services within the various components of a spatially structured farmscape. It also requires determining how the spatial arrangement of the components of the agroecosystems determines the efficiency with which these agroecosystem services are provided.

For example, pollination and pest control services depend on the interaction of the spatial arrangement of vegetation in the farmscape and on the traits that determine species' movements through the farmscape (e.g. dispersal, habitat preferences), meaning that farmscapes with spatial heterogeneity in vegetation types may have higher yields because pollinators and pest predators will be able to disperse across more of the farmscape's cultivated area (Ricketts et al. 2004, Garibaldi et al. 2011, Karp et al. 2013). Alternatively, pests may also rely on non-crop vegetation types to complete their lifecycles; therefore, understanding pest traits could additionally provide valuable insights into ecosystem disservices that may compromise farm yields (Chaplin-Kramer et al. 2011).

Practically, spatially explicit trait-based models of ecosystem services or disservices could be used to apply a trait-based approach across a complex landscape (Lavorel et al. 2011). These modeling approaches would first identify the landscape patches important to the provisioning of certain ecosystem services (Fahrig et al. 2011). Field sampling could then be used to measure the services in these patches, the traits of the ecosystem-service providing organisms, and abiotic properties that may impact those organisms. For instance, soil nitrate availability—a key resource for crop growth—can be measured in fields, hedgerows, and agroforestry plots as it relates to plant functional traits and abiotic properties.

Measurements of trait diversity and ecosystem services can also be georeferenced and used to calculate metrics of spatial configuration to determine how space influences functional trait control of ecosystem services (Cushman et al. 2008). For instance, Biswas et al. (Biswas et al. 2015) demonstrated that fine-scale responses of plant functional trait diversity to environmental disturbance exhibit greater unexplained variance and evidence of local-scale competition than coarse-scale patterns. Combining such spatial metrics with data on traits and

abiotic characteristics would allow for the development of spatially explicit models of ecosystem services that use point data to predict the landscape distribution of ecosystem services (Lavorel et al. 2011). Doing so will allow ecologists working in agricultural systems to identify the scale at which trait diversity responds to farm management decisions as well as the scale at which this trait diversity correlates with changes in environmental outcomes and ecosystem services. Models with and without trait data could then be compared to determine the importance of traits vis-à-vis environmental properties to particular ecosystem services.

Such a spatially explicit representation of traits and ecosystem services would also be important because functional traits—and associated services—can vary through the farmscape over time. For instance, plant matter of N_2-fixing plants is often relocated from one field to another to improve soil fertility. Sampling vegetation and soil nutrient status in single plots would fail to identify the effect of N_2-fixation on soil nutrient availability in the broader farmscape by ignoring this transfer of plant matter between farm fields.

1.3.2 Traits of multiple trophic levels

In addition to being focused at small spatial scales, most research on biodiversity-ecosystem functioning has been conducted at single trophic scales (Reiss et al. 2009). Yet the ecosystem services provided by agriculture often depend on activity within multiple trophic levels and interactions across trophic levels (Thompson et al. 2015). For example, rates of symbiotic N_2-fixation are determined by the activity of several trophic levels. Leguminous plants (level 1) regulate carbon and oxygen flow to roots that symbiotic N_2-fixing microorganisms (level 2) use to fix atmospheric N_2. Root-feeding nematodes (level 3) can suppress N_2-fixation by feeding on roots and decreasing the number of root nodules for N_2-fixation (Ibewiro et al.

2000). Similarly, for pest control, consumptive predator activity traits (level 1) affects pest populations (level 2), which in turn affect crop yields (level 3) (Letourneau et al. 2009, 2011).

A trophic, trait-based framework of ecosystem functioning requires quantifying the traits involved in species' responses to the abiotic environment, species' effects on the environment, and species' effects on and responses to the presence and activity of species at other trophic levels (Lavorel et al. 2013). Within a given trophic level, traits determine (1) the effect of that trophic level on an ecosystem process/service; (2) the response of that trophic level to higher trophic levels; (3) the effect of that trophic level on lower trophic levels (Lavorel et al. 2013). These latter two types of traits (i.e. "trophic traits") can inform how trait interactions across trophic scales may improve inference about the relationship between agrobiodiversity and ecosystem services.

1.3 Applications of a trait-based approach to agriculture

Important initial steps have already been taken to apply a trait-based framework to agroecosystems. The bulk of this initial research has focused on using traits to understand how biodiversity in agricultural systems responds to environmental conditions and land management. Some examples include weeds (Gaba et al. 2013), pollinators (Rader et al. 2014, Forrest et al. 2015), pasture vegetation (Fontana et al. 2014), soil macrofauna (Pelosi et al. 2014), and soil microbes (Wood et al. 2015b). Most work connecting species-based measures of biodiversity to agroecosystem services focuses on pollination (Garibaldi et al. 2013) and pest control (Letourneau et al. 2009, 2011). Research on the contribution of intercropping to productivity has largely focused on functional group classifications (Brooker et al. 2015). In a recent example, crops of broadly different functional types (legumes, fruits, and vegetables) were planted in different combinations and shown to increase production (Franco et al. 2015). Some initial work

has also applied functional group classifications to pollination and pest control services. The diversity of functional groups of bees (based on flower height preference, time of flower visitation, and body size) explained more of the variance in pumpkin seed set than did species richness (Hoehn et al. 2008). For pest control, functional group diversity of birds (classified into functional groups based on body mass, foraging strategy and strata, and diet) was a significant predictor of arthropod removal (Philpott et al. 2009). However, in contrast with findings from a pollinator system (Hoehn et al. 2008), bird functional group diversity was not as strong of a predictor of ecosystem services as species richness.

Less work has considered how continuously varying measures of functional traits influence ecosystem services. Studies that do link continuous measures of functional traits to ecosystem services in agricultural systems are mostly based in experimental grasslands and are framed in an ecosystem service context through forage production (Laliberté and Tylianakis 2012, Gardarin et al. 2014). For instance, Laliberté and Tylianakis (Laliberté and Tylianakis 2012) show that resource addition and grazing strongly determine grassland functional trait diversity, which cascades to induce changes in grassland productivity, decomposition, and soil carbon sequestration. Abiotic and biotic factors directly impacted functional diversity, directly impacted ecosystem functioning, and indirectly impacted ecosystem functioning through changes in functional diversity. Wood et al (Wood et al. 2015a) applied a similar approach to soil microbes on African farms and showed that although microbial functional diversity can be strongly structured by farm management (Wood et al. 2015b, 2015a), functional diversity is a weaker predictor of ecosystem processes than abiotic factors. This approach that simultaneously assesses the influence of biotic and abiotic controls allows ecologists to determine when functional diversity is a key control on agroecosystem services.

1.4.1 Traits across spatial scales

Much of the application of trait-based research to agriculture has focused on small spatial scales. For instance, Doisy et al (Doisy et al. 2014) show that the functional traits of weed seeds and cover crop grasses at the plot level are key predictors of weed seed interception by grasses that prevent weed establishment. Other research in weed science has demonstrated the key role traits play in weed population persistence and interaction with crop production (Navas 2012). In grassland and cropland plots, root traits are strongly correlated with improved soil physical and biological properties at the local level and are important factors that allow grasslands to maintain productivity (DuPont et al. 2014).

While this trait-based work has significantly advanced understanding of local effects of and controls on agrobiodiversity, few studies have been done at larger spatial scales. In one of the few studies at a larger spatial scale, Remans et al (Remans et al. 2014) show that nutritional functional traits of crops are an important predictor of nutrition-related health outcomes of national food systems. For animal nutrition, leaf dry matter content can be an important predictor of forage digestibility across climate conditions and management regimes (Gardarin et al. 2014). In pollinator systems, sociality (a key pollinator trait) is a strong predictor of pollinator response to landscape fragmentation (Steffan-Dewenter et al. 2002). Such landscape fragmentation, and resulting distance between pollinator habitat and crops, can have significant negative impacts on yields (Ricketts et al. 2004, Garibaldi et al. 2011). Because traits determine the movement of species through a landscape—as well as their effect on the landscape—more research is needed to understand how a community's influence on ecosystem services scales up to the landscape (Box 2).

1.4.2 Traits of multiple trophic levels

Trophic scale can be crucial to understanding agroecosystem services because many services provided by agriculture are determined by activity within and interactions across multiple trophic levels (Thompson et al. 2015). Storkey et al (Storkey et al. 2013) is one of the only studies to apply a trait approach to multiple trophic levels in arable systems. They show overlap in the response traits that govern plant response to regular plowing and the effect traits that impact the abundance of phytophagous invertebrates. Plant communities characterized by ruderal traits (e.g. high specific leaf area, early flowering) were also associated with greater invertebrate abundances, suggesting that growth strategy (e.g. ruderal vs competitive) can be linked to plant response to abiotic environment and other trophic levels.

1.5 Using traits to generate agroecosystem management strategies

Though functional traits can describe the mechanisms by which agrobiodiversity influences ecosystem functioning, farmers generally manage agroecosystems by directly manipulating the abundances and location of species or through physical and chemical manipulation of the agroecosystem (e.g. tillage, fertilization). Managers use traits implicitly by selecting or promoting species that have certain functional properties (e.g. able to fix N_2, attracts beneficial insects). Managers, however, do not often base management on explicit, quantifiable goals for functional trait distributions, such as the distribution of pollinator body sizes. Yet on functional trait distribution goals offer an opportunity to create management strategies tailored to environmental conditions and biotic interactions when the relationship between species, their traits, and the environment is well understood. Previous efforts to integrate functional trait research into ecosystem service assessments have been proposed, but these have stopped short of creating tangible management targets that can be practically implemented by managers (Díaz et al. 2007, 2011, Navas 2012). Because farmers manipulate species, not traits, effective

management strategies require understanding how trait response to the environment can be translated to species relative abundances targets. Farmers could then manipulate the biological, physical, or chemical components of agroecosystems to achieve species abundance targets (Laughlin 2014).

Management targets could be generated through quantitative trait-based modeling that converts functional-trait based objectives into targets for the relative abundances of species (Figure 2). In this way, data on functional traits of a local species pool could be used to determine the relative abundance of species needed to achieve a functional trait goal. A management strategy could then be implemented to try to achieve this relative abundance and then to test if the implemented community meets the established functional trait goals and the delivery of the desired ecosystem services (Laughlin 2014).

For planned diversity, establishing communities with certain relative abundances is relatively straightforward (e.g. planting legumes in a certain density to achieve soil nutrient goals). For associated diversity, which depends on ecological processes embedded in an agricultural setting, establishing and maintaining communities requires understanding how species respond to the specific management practices used; for example, how pollinator abundances respond to the presence of certain types of planted vegetation. Several approaches have been proposed, for example, to increase the abundance of pest enemies, including habitat modification and food supplementation (Chaplin-Kramer et al. 2011). However, it has been difficult to empirically assess how these factors actually contribute to the balance of natural enemies and pests and, thus, the level of pest control (Bianchi et al. 2006, Chaplin-Kramer et al. 2011) and resulting differences in crop yields.

Given the importance of space and trophic position in determining agroecosystem services, trait-model iterations of management targets ought to be applied to specific spatial and trophic scales. Because the implementation of these targets is iterative (e.g. develop ecosystem-service targets, apply management practices, assess if targets were met, implement new practices, etc.), it will be important to also consider how the properties of species and ecosystems change over the course of implementation (e.g. through time).

1.6 Concluding Remarks

Ecologists and agricultural scientists should join efforts to apply a trait-based framework to agrobiodiversity. Doing so will help generate a more predictive understanding of how agroecosystem services vary with farm practices and environment and help generate management strategies that can be implemented by farmers to manage agricultural systems to provide multiple ecosystem services. We propose a trait-based approach to agriculture that adopts recent advances in trait research for multi-trophic and spatially heterogeneous ecosystems. This approach should measure changes in the values of functional traits across environmental gradients and under different management scenarios, as well as at varying levels of complexity, such as across trophic positions and space. The resulting trait information can be paired with quantitative modeling approaches to generate specific agricultural management targets to manage agroecosystems to increase multiple ecosystem services (Box 1). Components of agrobiodiversity can also decrease ecosystem services, thus it is important to also quantify mechanisms controlling these "disservices" and trade-offs between them and services (Box 2).

Because trait-based research focuses on the multiple properties of species that determine their response to and impact on the environment, these approaches require more data than taxonomic approaches. If key traits are highly variable within species, measuring individual-

level trait values will be important across management systems and ecological zones. This will require greater expertise and technical resources than standard taxonomic efforts. It will thus be important to determine when in-depth sampling is needed (i.e. to determine intra-specific variation) and when sampling effort can be reduced. For instance, if important traits are constant within species, it could be possible to build trait databases for species and then predict ecosystem services by knowing which species are present, using previously recorded trait data (Box 2). To meet similar data needs in the broader field of ecology, advances have been facilitated by large-scale, coordinated collection and aggregation of trait data (Kattge et al. 2011, de Vries et al. 2012, García-Palacios et al. 2013, Adler et al. 2014). Ecologists working in agroecosystems should also establish a universally accessible agricultural trait database for all species in agroecosystems, across taxa, farm management, and environmental conditions. To do this, new data will need to be collected, but there is also much existing data collected by crop taxonomists, pest specialists, and agronomists on specific species. This fine-resolution approach will allow the more detailed mechanistic understanding of agrobiodiversity that will allow ecologists to design ecological agricultural management strategies needed for the sustainability of agroecosystems.

1.7 Glossary, Boxes, and Figures

1.7.1 Glossary

Agrobiodiversity: the diversity of organisms living in landscapes that are under agricultural management. **Planned agrobiodiversity** refers to the organisms directly chosen in the process of land management (e.g. crops, managed pollinators, etc.), while **associated diversity** is the diversity that persists in agricultural settings, but is not directly chosen (e.g. soil biota, wild pollinators, natural pest enemies, etc.). Planned agrobiodiversity is determined by political,

social, and economic factors; associated diversity is governed by ecological processes that allow these organisms to persist in agricultural settings.

Agroecosystem: an ecosystem, including biotic and abiotic elements and their interactions, that is managed for agricultural production. Agroecosystems can be low in biological diversity, such as monoculture farming in the American mid-west, or high in diversity, such as tropical forest gardens.

Ecosystem multifunctionality: the notion that ecosystems are composed of multiple properties, processes, functions, and services. Ecosystems can be managed to optimize the number and or magnitude of these functions or services. The concept was originally developed to illustrate that the effect of biodiversity on ecosystem functioning is greater when considering multiple functions because different species impact different functions.

Ecosystem service: a property or process in an ecosystem that confers either direct or indirect benefits on human beings. We focus on the goods that are directly used by people (e.g. food, fuel, and fibre) and the ecological processes that influence the provision of these goods (e.g. pollination, soil nutrient cycling, etc.).

Facilitation: the presence of one species enhances the functional contribution of another species, resulting in greater aggregate system productivity of functioning (Flombaum et al. 2014).

Farmscape: a landscape that is dominated by agricultural activities.

Functional diversity: the diversity of functional traits, rather than species or taxonomic units, in an ecological unit such as a plot, landscape, or ecosystem. Functional diversity influences ecosystem functioning directly, through effect traits, and indirectly, through response traits that determine species distribution patterns and, therefore, greater productivity through the effect traits of those species.

Functional trait: a property, either categorical or continuous, of an individual organism that determines its effect on (**effect trait**) or response to (**response trait**) the environment. Though a property of an individual, functional traits are often compared among species. Because of the empirical challenge in measuring traits for all individuals, **functional groups** are often used, such as body size classes. This approach does not capture often-important intraspecific variation, but can be more mechanistic than taxonomy-only approaches.

Niche complementarity: a mechanism for the effect of biodiversity on ecosystem functioning in which the diversity of co-occurring, functionally distinct, species increases overall efficiency of resource use and overall productivity. Niche complementarity is an aggregate of **resource partitioning** and **facilitation** (Flombaum et al. 2014).

Resource partitioning: a mechanism for biodiversity-ecosystem functioning in which different species use different resources and/or use resources in different ways, such that systems with a greater number of species will use a greater range of resource types and, thus, increase overall productivity (Flombaum et al. 2014).

Sampling effect: a mechanism for biodiversity-ecosystem functioning patterns in which increases in the number of species in a system increases the probability of including a species that is more productive than others, thus increasing overall productivity (Grime 1998, Flombaum et al. 2014). This is also known as the **dominant effect**.

1.7.2 Boxes

Box 1. How to implement a functional trait framework to agrobiodiversity.

A functional trait-based approach for agrobiodiversity consists of the following steps (adapted from Naeem and Wright (Naeem and Wright 2003)).

1) *Identify the components of the agroecosystem*. An agroecosystem is composed of multiple elements that are determined by abiotic properties—such as farm parcels on different soil types, aspects, and slopes—or agro-functional properties—such as the principle production type of an area (e.g. mixed maize cropping).
2) *In each identified element, identify the biotic composition and the broader species pool.*
    a. For associated diversity, determine community assembly mechanisms by applying environmental filter algorithms to regional species pools (Laughlin 2014). These community assembly mechanisms depend on species' response traits. This will inform which factors determine the abundance of species that make up the associated diversity.
    b. For planned and associated diversity, note the periodicity of turnover. Is the system dominated by crops that are present for a single growing season? Are cover crops used in the off-season? Do perennial biota exhibit phenological patterns? This will determine the temporality of sampling needed to measure biodiversity and ecosystem services at the relevant temporal scale.
3) *In each element, determine the abundance of relevant taxa*.
4) *Determine and measure the functional traits related to the function(s) or service(s) of interest*. Different functional traits are important for different services and ecosystem processes. These traits can be strongly impacted by agricultural management (Figure 1;

Box 2). The number of functional traits measured can strongly influence measurements of functional diversity (Maire et al. 2015). Determine and measure the relevant functional traits for the different taxa in the different components of the system and for the ecosystem processes of interest. For plant traits, use a guide to select traits and determine standard measurment (Pérez-Harguindeguy et al. 2013). Calculate metrics of functional trait composition (mean values or diversity, depending on the service of interest (Schleuter et al. 2010)).

5) *In each identified component, determine the ecosystem function or service of interest*. Determine which ecosystem service (s) is/are of interest and measure them at the scale of steps (1), (2), and (3).

6) *Compare the diversity and ecosystem function(s)/service(s) of agroecosystems to (agro)ecosystems they replaced, are likely to replace, or could be replaced by (Box 2).* An important step in understanding the functional consequences of agrobiodiversity is assessing tradeoffs in ecosystem services when habitats are transformed to or from agriculture or managed in new ways. Assessing the functional trait diversity within a given farmscape on its own may contribute to understanding of ecosystem service provision in that farmscape, but could miss the tradeoffs that occur when land is managed either as agroecosystems (e.g. croplands and livestock), different types of agroecosystems, or non-agroecosystems (e.g. prairie grassland with bison).

7) *Use modeling to generate target distributions of species based on their functional traits*. Quantitative modeling approaches can be used to convert targets for functional traits to specific management goals based on the relative abundances of species (Laughlin 2014) (Figure 2). This procedure is implemented iteratively to make sure that management

strategies to achieve specific relative abundances successfully achieve functional trait targets and that those functional trait targets successfully achieve goals for the rates of ecosystem processes and services.

Box 2. Outstanding questions.

Which traits determine the scale at which ecosystem services will be provided? Agroecosystem services can be provided at different scales. For instance, pollination occurs at the plant level, but the service is distributed across a farmscape or landscape. Dispersal- and habitat range-based traits interact with landscape composition and configuration to determine whether services are provided at local- vs. broader-scales. For instance, pollination depends on sociality; social pollinators are less impacted by landscape fragmentation than solitary bees (Steffan-Dewenter et al. 2002). More research is needed to understand what traits determine how organismal influence on ecosystem services scales up to the landscape.

What is the interaction among trait-based mechanisms for ecosystem services? Many ecosystem services are determined by separate, but simultaneously occurring mechanisms operating at different scales. Pest control, for instance, can be impacted by: (1) field environmental conditions, such as microclimate, that determine pest habitat suitability; (2) predator habitat suitability; (3) landscape factors impacting pest or predator dispersal; (4) direct predation on pests; (5) other sources of food for predators allowing them to build or maintain populations when pests are not abundant. These factors, which depend on response traits of pests and both response and effect traits of predators and vegetation, occur simultaneously and vary across environmental gradients. More research is needed to understand the factors that determine when certain mechanisms are dominant and when and how they interact.

How does functional diversity's influence over ecosystem function and services in natural systems compare to agricultural systems that have replaced them? Applying management approaches to agriculture requires comparing existing systems with other possible states in order

to create target goals. How does agrobiodiversity in current agroecosystems compare to systems that they replaced or systems that could be implemented in their place?

Can farmscape or landscape diversity substitute for plot-level diversity? Highly diverse intercropping or field management systems can be too labor intensive to be feasible. Similarly, allowing part of a farmscape to regenerate wild vegetation can represent economic losses to a farmer. Regional-scale exchanges in nutrient flows between farms depends on the diversity of farm types locally (Nowak et al. 2015). Can farm type diversity across a landscape substitute for local-scale diversity in terms of its effects on ecosystem services? Because functional traits determine how species move through a landscape, a functional trait approach is key to understanding the spatial substitutability of agrobiodiversity. Chaplin-Kramer and Kremen (2012) show that local- and landscape scale- complexity may be somewhat substitutable for pest control services.

Are important functional traits common across taxa? Several key functional traits (e.g. body size) are shared across taxa, ranging from soil fauna to pollinators to pest control agents. To what extent are these common traits equally important to the services provided by each group of taxa? Is there a set of core traits that can be measured across trophic levels to provide an informative understanding of the ecosystem services in a given agroecosystems?

Are the most important traits plastic or rigid? Certain traits are more important than others in determining the distribution and impact of agrobiodiversity in agroecosystems. These important traits could either be highly variable (e.g. plastic) or constant (e.g. rigid) within a species. If they are rigid, would it be possible to build trait databases for species and then predict ecosystem services by knowing which species are present? Or is there enough interspecific variation that we need to measure traits in every context if we want to predict ecosystem service outcomes?

How do individual species and their functional traits respond to specific management strategies? A core component of implementing trait-based management strategies is developing an understanding for how species respond to particular management techniques. If the goal is to create a community of pollinators, for instance, with a particular distribution in body size, then there needs to be clear understanding of which management strategies will be successful in establishing such a community.

How does understanding of the relationship between functional diversity and stability relate to resiliency and food security under environmental and social change? A key area of research in biodiversity-ecosystem functioning is how diversity can minimize the variability of ecosystem processes through time. Minimizing the variability of agricultural production would be a key service, especially under environmental and social change. Past research has suggested that maintaining a high diversity of response traits within functional groups (pollinators) is a key mechanism to increasing the resilience of services provided. Do the same principles of diversity-stability apply to agrobiodiversity-resiliency? What are the conditions under which these principles apply and the conditions under which they do not?

Quantify disservices as well as services. Ecosystems provide benefits, but some components of biodiversity can also negatively impact human well-being (Zhang et al. 2007). In agriculture, crop pests provide a disservice, rather than a service, because they decrease crop production. However, such pest outbreaks may be a result of system simplification and the inclusion of diverse pest predators may control such disservices. When does biodiversity lead to services and when does it lead to disservices?

1.7.3 Figures

Figure 1. **Potential trajectories of functional trait space across a land-use trajectory, from natural system to low-impact use, high-impact use, and restoration.** Trait space represents combinations of three hypothetical traits. Greater trait space hypothetically corresponds with greater functional capacity, though the relationship between trait space and functional capacity is more complicated in reality (Albert et al. 2010a). Conversion to monoculture can lead agroecosystems to be depauperate in trait space (1. degraded), but should also be able to contain trait assemblages that are recovering (2. recovering) or functionally similar to natural systems (3. baseline; Lin et al. 2011). Human-constructed assemblages may also exceed trait space of baseline conditions by including species evolved in other contexts that possess different functional qualities (4. improved). Movement along the x-axis is not necessarily temporal.

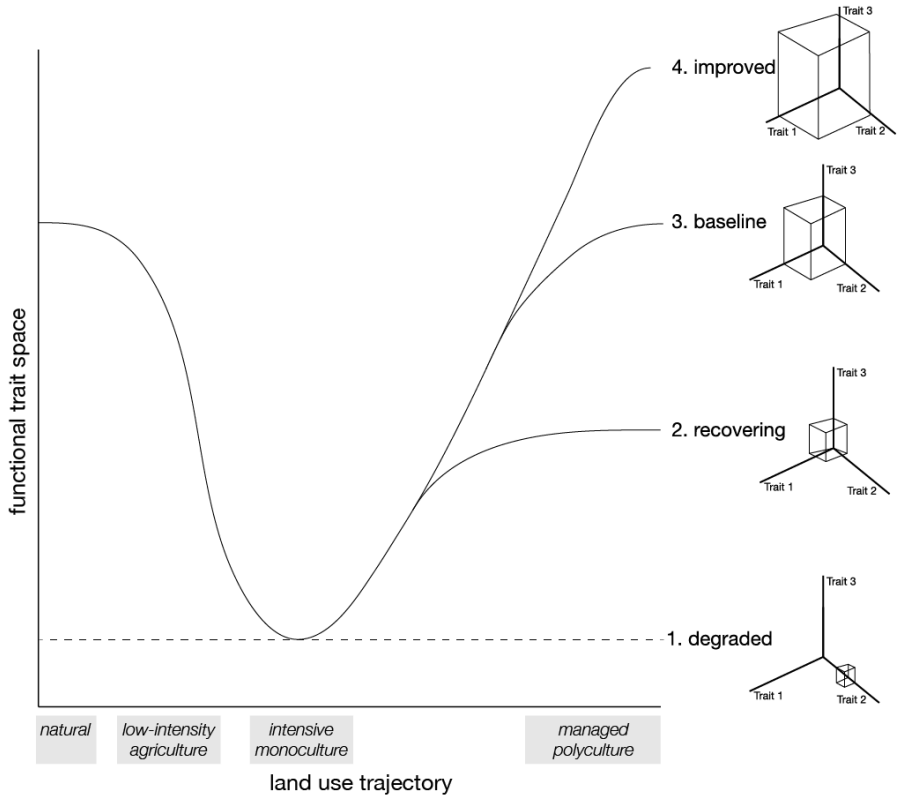
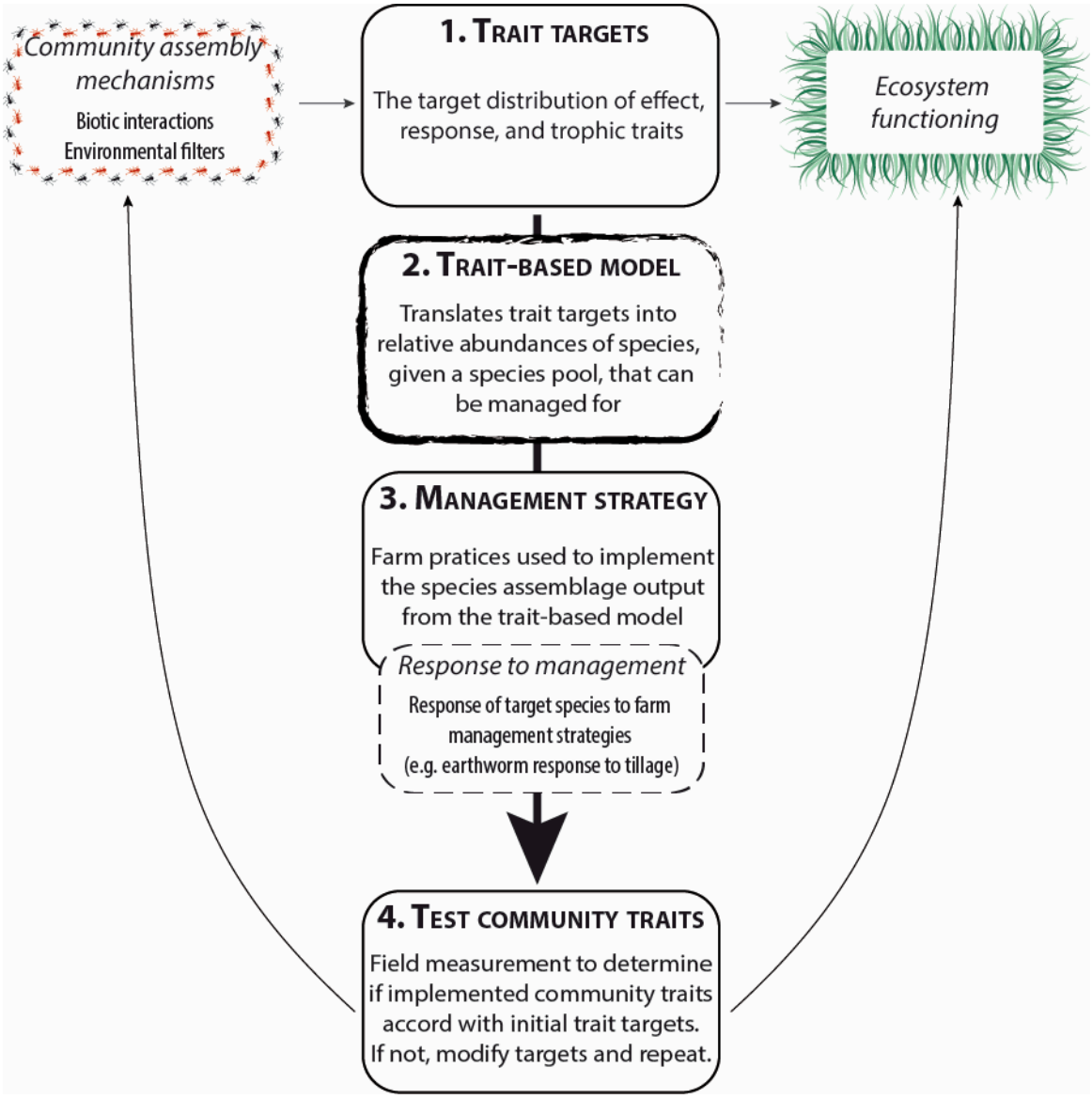


Figure 2. **A trait-based modeling approach to translating functional trait targets into farm management strategies.** Empirical understanding of response and effect traits of present species is used to create target trait distributions (1). A trait-based model converts these targets into relative abundances of present species (2). A farm management strategy is applied to generate these relative abundances (3), which requires understanding the response of species to management (Box 2). Traits of the implemented community are measured to test if they fit with original goals for traits and functional outcomes. Adapted from Laughlin (2014).



CHAPTER 2. AGRICULTURAL INTENSIFICATION AND THE FUNCTIONAL CAPACITY OF SOIL MICROBES ON SMALLHOLDER AFRICAN FARMS

Published as:

Wood, S.A., Bradford, M.A., Gilbert, J.A., McGuire, K.L., Palm, C.A., Tully, K.L., Zhou, J., and Naeem, S. 2015. Agricultural intensification and the functional capacity of soil microbes on smallholder African farms. Journal of Applied Ecology: 52, 744-752, doi:10.1111/1365-2664.12416.

2.1 ABSTRACT

1. Fertilization may impact ecosystem processes that sustain agriculture, such as nutrient cycling, by altering the composition of soil microbial communities that regulate such processes. These processes are crucial to low-input, smallholder tropical agriculture, which supports 900 million of the world's poorest people. Yet little is known about how efforts to increase crop yield on such farms will affect the capacity of soil microbial communities to carry out ecosystem processes.

2. We studied the diversity and functional capacity of microbial communities on smallholder farms in western Kenya. We measured functional capacity as the abundance of functional genes involved in several components of nutrient cycling as well as catabolism of multiple carbon substrates; taxonomic diversity was measured using metagenomic sequencing. Diversity and functional capacity were measured on short-term, experimental mineral fertilizer addition plots and on actively managed farms that have maintained for at least seven years a

management strategy of low mineral fertilization, high mineral fertilization, or high fertilization combined with legume rotations.

3. Soil bacterial diversity decreased with mineral fertilizer addition, with a community shift towards taxa that thrive in high-resource conditions. This taxonomic response did not correspond with decreased microbial functional capacity. Instead, functional capacity was increased, along with yields, when fertilizers were combined with legume rotations that add organic matter to soil.

4. *Policy implications.* Mineral fertilizer use is associated with lower soil microbial diversity on smallholder farms, but not associated with changes in microbial functional capacity. Functional capacity is highest, along with yields, when mineral fertilizers are paired with legume rotations. Our findings suggest that this type of agroforestry can be an important strategy for maintaining the long-term functional capacity of soil microbes as well as increasing crop yields on smallholder farms. These observations support proposals to achieve long-term food production targets in sub-Saharan Africa by combining mineral fertilizers with organic inputs.

2.2 INTRODUCTION

Intensive agriculture has driven increases in crop production, but is responsible for environmental damage, such as water pollution and greenhouse gas emissions (Vitousek et al. 2009). Intensification may also impact the ecosystem processes that sustain agriculture, such as soil nutrient cycling, by altering the composition of soil microbial communities that mediate these processes. The composition of microbial communities is controlled by land management, such as fertilizer addition (Ramirez et al. 2010, 2012, Fierer et al. 2012), yet little is known about

whether management-induced changes in community composition will feed back on microbial capacity to control the ecosystem processes on which agriculture depends.

Soil nutrient cycling processes, such as carbon (C) and nitrogen (N) cycling, are especially crucial to low-input smallholder tropical agriculture, which supports 900 million of the world's poorest people on 500 million farms of less than 2 ha (Wiggins et al. 2010). Nutrient budgets on these farms are undergoing rapid change due to increases in mineral (Vitousek et al. 2009) and organic fertilizer use (Glover et al. 2012) promoted to increase yields and decrease poverty – often referred to as the African Green Revolution. It remains unknown how such modifications to nutrient economies of smallholder farms will impact the functional capacity of soil microbial communities.

Fertilization decreases the diversity of plant communities (Bobbink et al. 2010) and causes shifts in microbial community composition (Ramirez et al. 2010, 2012, Fierer et al. 2012). We thus hypothesize that fertilization on tropical smallholder farms will be associated with decreased microbial taxonomic diversity and a shift in community composition towards taxa that perform well in high-resource environments (e.g. copiotrophs), thus resulting in lower microbial functional capacity (*diversity-functioning hypothesis*; (Bell et al. 2005)). Lower taxonomic diversity should lower functional capacity by creating a community that has a lower range of functionally distinct taxa in similar abundances. A community shift towards copiotrophic taxa should also decrease functional capacity by producing a community with a lower ability to use recalcitrant C that makes up the bulk of the soil C pool and, thus, lower heterotrophic respiration and microbial standing biomass (Fierer et al. 2012), which is a key control of microbially mediated processes.

Microbial functional capacity is also constrained by nutrient availability (Drake et al. 2013). We therefore alternatively hypothesize that fertilization will increase functional capacity by allowing microbes to overcome nutrient limitation and thus increase their potential contribution to ecosystem processes (*limitation release hypothesis*). The addition of mineral and organic nutrients, which co-limit microbial activity, should build functional capacity by increasing the ability of microbes to produce extracellular enzymes that drive organic matter decomposition (Drake et al. 2013). As a result, soil microbes should increase their active biomass (Drake et al. 2013). If the *limitation release hypothesis* is supported then we would expect microbial functional capacity to increase with fertilization, along with fertilizer-induced increases in crop productivity.

To test these hypotheses, we collected data on microbial taxonomic diversity and community composition, functional capacity, and crop yield from experimental plots and actively managed farms in western Kenya (Figure 1). Actively managed farms are categorized as low fertilizer use, high fertilizer use, and high fertilizer use plus legume rotations. Experimental plots only include mineral fertilizer addition. The study was conducted in the Sauri village cluster of the Millennium Villages Project (MVP) in western Kenya (Figure 1). The MVP agriculture strategy aims to implement an African Green Revolution strategy through high-yielding crop varieties, mineral fertilization and combining fertilizer use with organic inputs.

2.3 Materials and Methods

The study zone is mixed maize agriculture with maize production usually occurring twice annually, during a long rainy season (March–June: 1100 mm) and a short rainy season (September–November: 700 mm). Soils are Kandiudalfic Eutrodox (U.S.D.A) and are well-drained sandy clay loams derived from volcanic parent materials.

We sampled soil from both a controlled fertilizer addition experiment and actively managed farms with at least seven years of low or high fertilizer use or high fertilizer use paired with seasonal legume rotations. On experimental plots, we sampled from five levels of mineral fertilization (0, 50, 75, 100 and 200 kg N ha^{-1}). Each treatment has four replicates. Plots are 6 × 3 m and arranged in two rows, separated by 0.5 m within a row and by a 10 m buffer between rows. Fertilizer is added in a split application with one-third added at planting as diammonium phosphate and the remainder added as urea at top-dressing (4–6 weeks after planting). Because diammonium phosphate is 18% NH_4 and 46% P_2O_5, which is 44% P, the fertilization treatment also adds: 0, 3.35, 5.02, 6.69, and 13.38 kg P ha^{-1}. This management was maintained for two years prior to sampling, before which the land was unplanted fallow. There is no legume rotation treatment on the experimental plots. Experimental plots are located approximately in the middle of the study zone at 0°06’04.88 N, 34°30’40.12 E at an elevation of 1450 m (Figure 1).

Actively managed farms were selected to represent three broad management approaches: low fertilizer, high fertilizer, and high fertilizer + legume rotation. In the long rainy season, high fertilizer farms received 60 kg N ha^{-1} or more, but often closer to 60 kg N ha^{-1} since this is the recommended application amount, and low fertilizer farms received less than 10 kg N ha^{-1} (Table 1). On legume rotation farms, farmers replace short-rain maize crops with fast-growing leguminous tree, shrub, or herbaceous species (Table 1) that are planted from seed and cut each year for organic inputs to crop fields. These legume rotation techniques were initially promoted in Sauri in the early 1990s as a low-cost option for improving soil fertility.

Farm selection was based on two years of household surveys on 42 candidate farms to identify N inputs (from reported inputs of diammonium phosphate, calcium ammonium nitrate, and urea), maize yield, and crop choice over the past 10 years. The 21 farms included in the final

list reported management strategies that were not highly variable over the 10-year reporting period, had inputs and outputs that roughly agreed, and had farmer-reported yields that were demonstrative of their reported fertilizer levels. Based on reported N_2-fixation rates in the region, we conservatively estimate that N_2-fixation contributed between 30 to 50 kg N ha^{-1} $year^{-1}$ (Gathumbi et al. 2002a). Planting densities can vary widely from year to year with low-density years being as low as an order of magnitude less than those assumed in this estimate. Thus actual fixation rates may be as low as 5 to 30 kg N ha^{-1}.

In the final farm sets all treatment types were clustered spatially into *farm sets* (Figure S1) to control for differences in elevation and texture across the landscape. Farms in a set are all situated within 200 m of one another along the same contour or slope. Sample size was limited by the fact that farmers that live in close proximity tend to have similar farming approaches. To partially address this issue of sample size, we added pairs of high and low fertilizer farms, which was a more common treatment than long-term legume rotation. The final list includes 21 farms grouped into five sets of the three farm types plus three high-low pairs, totalling eight high fertilizer farms, eight low fertilizer farms, and five legume rotation farms.

Soil sampling was conducted in June 2012, in the middle of the long rains, two weeks after fertilizer application. On the farm fields, we took 15 2-cm diameter soil cores from the top 20 cm of bulk soil. Cores were taken at regular intervals throughout the entire farm field and homogenized and aggregated to a composite sample. Because experimental plots were significantly smaller than farms (18 m^2 compared to 0.1–3.7 ha), we took nine 2-cm cores per plot and aggregated to a composite sample. Soils were sieved to 2 mm using a UV-sterilized sieve. Soils for catabolic assays were immediately refrigerated and transported to the lab within one week of sampling where they were stored at 4 $^{\circ}$C. Soils for DNA extraction were

immediately frozen and transported to the lab within one week of sampling where they were stored at -20 °C.

A subsample of sieved soil was air-dried and used to determine total C and total N by combustion with an Elementar Vario Macro CNS analyser. Extractable P and micronutrients were assessed by inductively coupled plasma spectrometry (Varian Vista MPX Radial ICP-OES). Soil texture was determined using the standard hydrometer method. Yields were measured by harvesting aboveground biomass in a 3 × 3 m subplot on actively managed farms and by harvesting the entire plot on the experimental farm, less the border rows. Harvested plants were separated into stalks and cobs and weighed in the field. Subsamples were taken from the field, cobs separated into core and grain, and all materials weighed fresh and oven-dried (60 °C until constant mass was obtained). Plot yields were estimated based on dry grain per plant and the total number of plants per plot.

Subsamples of sieved field soil (stored at 4 °C for one month) were used to determine pH, gravimetric soil moisture, and water holding capacity using standard methods. Active microbial biomass was determined using modified substrate-induced respiration (West and Sparling 1986). Microbially available C was estimated using a 30-day C mineralization assay (Bradford et al. 2008) by measuring CO_2-efflux across thirty days (days 1, 4, 15, 30). For each measurement, 4 g of soil were placed in 50-mL centrifuge tubes that were fitted with gas-tight lids. Tubes were flushed with CO_2-free air and incubated for 24 h. Headspace CO_2 concentrations were measured using infrared gas analysis (Li-COR model LI-7000, Lincoln, NE, USA). Samples were maintained at 60% water holding capacity across the 30-day period.

To classify soil bacterial communities, we extracted DNA, amplified the 16S rRNA V4 gene, and sequenced the gene using an Illumina MiSeq instrument at Argonne National Laboratory (Gilbert et al. 2010). The 16S rRNA gene is a well-conserved gene in bacteria that captures evolutionary relationships among bacterial taxa. Sequence reads were binned into operational taxonomic units (OTUs) based on a 97% similarity threshold. OTUs were then compared to GenBank to identify bacterial lineages. All procedures were performed using the standard protocols of the Earth Microbiome Project (www.earthmicrobiome.org/emp-standard-protocols/; Gilbert et al. 2010). A total of 3 462 835 bacterial sequences were generated across all samples, representing 29195 OTUs. Sequence lengths averaged 150.63±2.93 per sample; samples were compared at a depth of 40 sequences per sample.

To assess the abundance of key functional genes, we used GeoChip 4.0 to analyse DNA samples that were extracted following the protocol for taxonomic assessment. GeoChip is a functional gene array that examines the abundance of thousands of functional gene variants simultaneously through a fluorescent procedure. DNA samples were labelled with a fluorescent dye and purified following Yang et al (2013). Labelled DNA was suspended in a hybridization solution before hybridization on a MAUI station (BioMicro, Salt Lake City, UT, USA). GeoChip microarrays were scanned by a NimbleGen MS200 scanner (Roche, Madison, WI, USA). Signal intensities were quantified and processed using a previously described data analysis procedure (Yang et al. 2013). We analysed: ammonification, assimilatory N reduction, C fixation, cellulose, chitin, hemicellulose, lignin, pectin, and starch degradation, denitrification, dissimilatory N reduction, methane oxidation, methane production, N fixation, N limitation, nitrification, phosphate limitation, and phosphorus utilization. Some categories are aggregates of specific genes, such as 'denitrification,' which includes *narG*, *nirK*, *nirS*, *norB*, and *nosZ.*

We assessed the ability of microbial communities to degrade C substrates using a catabolic profiling assay that measures microbial respiration on a range of C substrates that represent key plant inputs to the soil system, including root exudates (labile) and structural parts of plants and fungi (recalcitrant) (Degens and Harris 1997). Included substrates are sucrose, glucose, glycine, citric acid, oxalic acid, yeast, chitin, and cellulose. 8 mL of each of the eight different substrates (plus a control) were added separately to 4 g of soil (dry wt equivalent) in a 50-mL centrifuge tube to make a slurry and shaken for 1 h. Soils were capped and flushed with CO_2-free air and incubated at 20 $^{\circ}$C for 4 h (labile substrates) or 24 h (recalcitrant substrates). Net CO_2 production was measured by injecting 5 mL of centrifuge headspace into an IRGA (Li-COR model LI-7000, Lincoln, NE, USA).

We calculated Shannon diversity of OTUs and Faith's phylogenetic diversity (PD) from unweighted UniFrac differences in OTUs among samples (Lozupone et al. 2011). Faith's PD constructs a phylogenetic tree and calculates the sum of all branch lengths in the portion of the tree connecting a given set of OTUs. For experimental plots, we fitted visually weighted regression models of changes in microbial diversity and community composition. Visual weighting adjusts the colour saturation and contrast of bootstrapped regression lines proportional to an estimate's variance; ranges of the data where the confidence interval is dark and sharply contrasted with the regression line indicate high confidence in that local data region (Hsiang 2013). Since this approach is non-parametric and cannot be used for hypothesis testing, we used piecewise linear regression for hypothesis testing (Muggeo 2003). Piecewise linear regression identifies thresholds in the microbial response to fertilization and fits separate linear regressions for the separate segments of the data.

For all models on experimental plots, we included fertilization treatment, soil pH, % C, and % N as control variables and selected a final model that optimized adjusted R^2. The terms 'control variables' and 'covariates' are interchangeable; we use the former to highlight that we are interested in the effect of farm management on microbial diversity and functional capacity, controlling for broad soil properties. We did not include texture as a control because of lack of variation among the experimental plots. We standardized independent model coefficients using a z-transformation that produces coefficients representing standardized slopes, which are comparable in magnitude within models because variables are expressed in common units (Schielzeth 2010). Because the response of community composition to fertilization on experimental plots was linear, we used a conventional linear modelling approach, rather than piecewise regression as used for the diversity variables.

For active farms, we fit generalized least squares models assuming a Gaussian error distribution. As on experimental plots, we included fertilization treatment, soil pH, % C, % N, and texture as control variables. Because of the spatial distribution of farms, we tested for spatial autocorrelation using Moran's I. When present, we controlled for autocorrelation by weighting residuals by the semi-variogram of autocorrelation; when not present, we used linear mixed effects (LME) models with farm set – the spatial cluster to which each farm belongs – as a random effect (Bates et al. 2012). The final LME models were selected to minimize AIC and adjusted R^2 values are reported as a measure of model goodness-of-fit (Tables 2, 3). The reported R^2 value represents the amount of variance explained only by the fixed effects and is calculated by adapting a previous approach for calculating non-adjusted R^2 values for LMEs (Nakagawa and Schielzeth 2013). The F-statistic is not considered valid for the "lme4" package (Baayen et

al. 2008); we therefore estimated *P*-values and coefficients following the Satterthwaite approach to estimating denominator d.f. (Kuznetsova et al. 2013).

To assess functional genetic capacity of GeoChip data we used the same modelling framework of community composition described above. To assess catabolic potential we applied an approach designed to assess multiple ecosystem processes (Byrnes et al. 2014); respiration of each substrate was considered analogous to a separate ecosystem process. Although this approach has been criticised when applied to processes that are individually important and have context-dependent underlying drivers (Bradford et al. 2014c, 2014b), this approach is well suited to determining the mean response of multifunctionality when individual processes, such as respiration of different C substrates, are not highly informative individually, but together broadly represent functional capacity. Our response variable was the number of substrates with respiration rates exceeding a given threshold of maximum respiration, for thresholds ranging from 5% to 99%. We calculated the maximum respiration rate for each substrate across all samples as the mean of the n+1 highest measurements, where n is the smallest sample size of a single treatment. To model the effect of farm management on catabolism, we used a generalized LME model with a quasi-poisson error distribution. This model was iterated for each threshold level between 5% and 99% to assess at which thresholds the relationship between treatment and catabolism is significant. For farm sets, models were fit with set identifier as a random effect. For all statistical tests, we considered coefficients with $P < 0.05$ significant and coefficients with $P < 0.10$ marginally significant (Hurlbert and Lombardi 2009).

2.4 RESULTS

On experimental fertilization plots, crop yield increased from 1.52±0.23 t ha^{-1} on control plots to 2.17±0.38 t ha^{-1} on plots receiving 200 kg N ha^{-1} (Table 4). On actively managed farms,

yield increased from 0.86±0.40 t ha^{-1} on low fertilizer plots to 2.67±1.22 t ha^{-1} on high fertilizer plots. The combination of legume rotation with mineral fertilizers increased yields further to 3.25±1.02 t ha^{-1} (Table 4). However, fertilizer and legume rotation management did not affect broad measures of soil quality, such as total C, N, and P (Table 4). The legume rotation treatment did increase a fine-resolution fraction of soil C, specifically microbially available C (Table 4), which is a proxy for the size of the labile C pool (Bradford et al. 2008).

Supporting the assumptions of the *diversity-functioning hypothesis* that fertilization will lower diversity, taxonomic diversity was significantly lower on fertilizer addition plots, with the strongest decrease occurring between 0 and 75 kg N ha^{-1} (Shannon: 2.15% decrease, $P < 0.05$; Faith's PD: 6.12% decrease $P < 0.1$; Figure 2; Table 5). We observed a qualitatively similar decrease on active farms receiving high vs. low fertilization (Shannon: 2.14% decrease, Faith's PD: 1.41% decrease; decrease NS; Figure 2; Table 5).

Supporting the assumption of the *diversity-functioning hypothesis* that fertilization will shift communities towards copiotrophic dominance, we found that *Gammaproteobacteria*, which broadly represent copiotrophic taxa (Ramirez et al. 2010, 2012), significantly increased in relative abundance with fertilization on experimental plots (117% from 0 to 200 kg N; $P < 0.01$) and with legume rotation (29%; $P < 0.1$; Figure 3 and Tables 2, 3). *Deltaproteobacteria* are broadly considered oligotrophic, and thus are expected to have greater relative abundance under low-resource conditions (Ramirez et al. 2010, 2012). Consistent with this, we found that the relative abundance of *Deltaproteobacteria* significantly decreased with fertilization (19% from 0 to 200 kg N; $P < 0.05$) and legume rotation (29%; $P < 0.05$; Tables 2, 3). We also found a 577% increase in the coefficient of variation (CV) of Shannon diversity and a 99% increase in the CV of Faith's PD between 0 and 200 kg N ha^{-1} (Figure 4 A, C). We also found a 271% increase in

the CV of *Gammaproteobacteria* and a 20% increase in the CV of *Deltaproteobacteria* between 0 and 200 kg N ha^{-1} (Figure 4 B, D).

Despite support for the assumptions of the *diversity-functioning hypothesis* (lower diversity, community shift towards copiotrophic taxa), we found little evidence that decreases in taxonomic diversity and altered community composition due to mineral fertilization were associated with altered functional capacity (Figure 5, Tables 6, 7). On experimental plots, there was no significant change in either the relative abundance of genes in key functional categories or measured C catabolism (Figure 5, Tables 6, 7). Legume rotation, by contrast, was nearly always a significant positive predictor of functional capacity (Tables 6, 7), consistent with the *limitation release hypothesis*. On legume rotation farms, genes related to C cycling, degradation, and fixation had significantly elevated abundances (Tables 6, 7). Legume rotation was often twice (or more) as strong of a predictor of C-related functional gene abundances as fertilizer use without legume rotation, as shown by standardized regression coefficients (Tables 6, 7). Legume rotation also contributed to the ability of microbes to catabolize a range of C substrates, with the contribution being greatest at highest thresholds of maximum catabolism (> 85%) and consistently less important at lower thresholds (< 50%; Figure 5).

The total abundances of genes related to N and P cycling were also significantly impacted by legume rotation (Tables 6, 7). Genes coding for denitrification, assimilatory N reduction, dissimilatory N reduction, and P use were significantly more abundant on legume rotation farms (Table 6). Genes coding for N_2-fixation were significantly less abundant with greater resources (Table 6). The inclusion of legume rotation practices had a greater relative impact on all N and P cycling genes than high fertilizer use, except for N_2-fixation genes, which were more impacted by fertilization (Table 6).

2.5 DISCUSSION

We found experimental mineral fertilizer use to significantly increase crop yields, but that the highest yield increases were observed when mineral fertilizer use was paired with legume rotation practices. These yield data support the proposal from proponents of an African Green Revolution that to maintain yields over time, mineral fertilizer use should be paired with inputs that increase soil organic matter (Glover et al. 2012). Consistent with previous work from smallholder African agroecosystems, fertilizer and legume rotation did not affect broad measures of soil quality, such as total soil C (Barrios et al. 1996). These observations are consistent with results suggesting that once particulate organic matter has decomposed (as would be the case in low-C, arable, sub-Saharan African soils), N addition has little effect on total soil C (Brown et al. 2014), although there may still be differences in specific soil C fractions. In support of this latter possibility, the legume rotation treatment increased a labile C pool (Table 4). The legume rotation treatment might then be expected to increase total C and N contents in the future, given that responses of these pools tend to be detected only over relatively long timescales (Conant et al. 2011).

We show that changes in microbial communities on smallholder farms in Kenya are predictable based on life-history traits (e.g. copiotroph vs. oligotroph). The specific taxonomic group responses we observed are also consistent with fertilization effects in temperate systems (Ramirez et al. 2010, 2012). Non-significant decreases in diversity with mineral fertilization on actively managed farms may be due to the fact that the range of fertilizer addition between the low and high fertilizer treatments (10–60 kg N ha^{-1}) is narrower than on experimental plots (0–200 kg N ha^{-1}), as well as year to year variability in environmental conditions and farm management.

We find that fertilization-induced losses in diversity and altered composition of microbial communities do not correspond with losses in the functional capacity of the soil microbiota, in contrast to what is predicted by the *diversity-functioning hypothesis*. Efforts to increase yield that combine mineral fertilization with legume rotation to build up soil organic matter have much stronger effects on microbial functional capacity than mineral fertilization alone. This suggests that legume rotation can be an important strategy for both increasing crop yields on smallholder farms and maintaining the long-term functional capacity of the soil microbiota. Our finding that the contribution of legume rotations to catabolic capacity was greatest at high thresholds of maximum catabolism suggests that the importance of legume rotation as a management strategy to promote microbial C use may depend on the level of catabolic capacity targeted.

In most cases, legume rotation was associated with higher abundances of genes related to C, N, and P cycling. In some cases, however, fertilization and legume rotation were associated with decreases in functional gene abundances. For instance, N_2-fixation genes were significantly lower with resource addition. Concurrent with this finding, symbiotic N_2-fixation in the tropics can down-regulate under high soil N conditions (Barron et al. 2011). Our results suggest that changes in microbial communities may help explain this down-regulation, though common explanations often focus on plant physiology (Arrese-Igor et al. 1999).

Because taxonomic diversity decreased with mineral N over the short and longer term, but functional capacity was only affected over the longer term (i.e. on farms and not plots), there appears to be a temporal decoupling between taxonomic and functional responses to mineral N addition. In other words, effects of nutrient addition on taxonomic composition emerge faster than effects on functional capacity. Commensurate with this apparent decoupling, we observed in the experimental plots an increase in the coefficient of variation of taxonomic diversity and

community composition between 0 and 200 kg N ha^{-1} (Figure 4). Theory and evidence suggest that increased variability in ecological communities can be an important precursor to shifts in alternative states (Scheffer et al. 2009). In our system, the observed increase in variability of diversity and community composition with fertilization over shorter time scales may, thus, help explain shifts in the functional capacity of communities that we observed over longer time scales.

Agricultural research has long focused on the direct influence of farm management on soil nutrient cycling processes. Evidence has begun to emerge that microbial communities can also act as an ultimate control of ecosystem processes. Bradford et al (2014b) show that microbial communities at local scales can act as a stronger control on decomposition than broad-scale factors that were previously thought to be dominant controls of nutrient cycling. Thus, management-induced changes to microbial communities may have important consequences for agroecosystem functioning. Research in temperate systems has shown positive correlations between taxonomic composition and catabolic capacity under fertilization (Fierer et al. 2012). In contrast, we find that in tropical smallholder agroecosystems that taxonomic changes under fertilization are not necessarily coupled with changes in functional capacity. Instead, functional capacity was generally increased, along with yields, when fertilizers were combined with legume rotation practices.

Our results demonstrate that legume rotation can be an important management strategy for both increasing short-term crop yields and building the ability of microbial communities to contribute to ecosystem processes that are crucial to agricultural sustainability over the long-term. This will be important for sustainable agriculture when enhanced microbial functional capacity – through, for instance, increased ability to decompose litter and convert nitrogen to

plant-available forms – leads to greater nutrient availability for crops. This increased functioning, paired with the additional N added from legume rotation, could reduce the need for farmers to invest in costly synthetic fertilizers when net N balances are positive.

Increased functional capacity could also play an important role in predicting changes in soil organic matter stocks. Because organic matter pools change over long time periods (Conant et al. 2011), indicators of the success of farm management to improve soil quality are needed at shorter, management-relevant time-scales. Increased soil microbial functional capacity may serve as such an indicator. While classical paradigms of soil organic matter turnover suggest that greater microbial functional capacity could deplete soil C pools through elevated mineralization of soil C to CO_2, emerging paradigms suggest that greater microbial activity may instead build up and stabilize soil organic matter pools (Schmidt et al. 2011). Increased functional potential may therefore be an indicator of soil quality and, as a result, crop production.

Trade-offs, however, may occur with increased microbial functional capacity, such as greater conversion of soil nutrients and organic matter to greenhouse gases. Future work will need to connect changes in microbial functional capacity with ecosystem process rates and to assess the potential trade-offs associated with these multiple processes (Wood et al. 2015a). Despite potential trade-offs, our findings support the notion that agricultural development strategies that are based on ecological principles, such as legume rotations, can both increase yields and build the capacity of the soil microbiota to contribute to soil nutrient cycling processes that are important to agricultural sustainability. Measures of microbial functional capacity might also serve as indicators of changes to soil organic matter stocks before actual changes are detected.

2.6 TABLES AND FIGURES

2.6.1 Tables

Table 1. Farm selection criteria for high fertilizer, low fertilizer, and high fertilizer + legume rotation farms.

	High Fertilizer	**Low Fertilizer**	**High Fertilizer + Legume Rotation**
Short Rain Activity	Maize	Maize	Legume Rotation
Management History	Fertilizer since 2005	Patchy fertilizer use	Annual fallow for at least last 7 years
N Amount	~ 60 kg N ha^{-1} yr^{-1}	< 10 kg N ha^{-1} yr^{-1}	65 - 110 kg N ha^{-1} yr^{-1}
Farm Area	Mean: 0.4 Min–Max: 0.1–3.7 ha	Mean: 0.3 ha Min–Max: 0.2–1.6 ha	Mean: 0.2 ha Min–Max: 0.1–1.3 ha
Species present			*Calliandra calothyrsus* *Crotalaria grahamiana* *Crotalaria paulina* *Crotalaria ochroleuca* *Mucuna pruriens* *Tephrosia candida*
Species Density			2000 plants ha^{-1}

Table 2. Drivers of relative abundance for the most abundant microbial groups on experimental plots. Relative abundances are expressed as percentages. Model coefficients were selected to maximize adjusted R^2. Model coefficients are standardized and thus represent the effect of a one standard-deviation change in the predictor variable.

Phylum	Class	Mean relative abundance on control plots (%)	Intercept	Fertilization (kg N ha^{-1})	pH	Carbon (%)	Nitrogen (%)	Adj. R^2
Acidobacteria		13.19	12.23****	-0.23		-1.25***		0.34
		(1.30)	(0.36)	(0.37)		(0.37)		
	Acidobacteria	2.79	2.79****	-0.14	-0.66****	-0.28**		0.71
		(0.75)	(0.11)	(0.12)	(0.13)	(0.12)		
	Acidobacteria 6	4.24	4.08****	0.07	0.50***	-0.47****		0.53
		(0.77)	(0.10)	(0.12)	(0.13)	(0.12)		
	Chloracidobacteria	2.17	1.87****	-0.01	0.16	-0.15		0.06
		(0.38)	(0.08)	(0.10)	(0.10)	(0.10)		
	Solibacteres	1.75	1.43****	-0.19**	-0.22**	-0.16*		0.42
		(0.44)	(0.08)	(0.09)	(0.09)	(0.08)		
Actinobacteria		17.82	19.56****	-0.01			1.08	-0.04
		(5.52)	(0.92)	(0.95)			(0.95)	
	Actinobacteria	8.55	9.75****	0.34			0.81	-0.04
		(4.34)	(0.73)	(0.75)			(0.75)	
	Thermoleophilia	7.95	8.62****	-0.05	0.48			-0.01
		(1.61)	(0.36)	(0.40)	(0.40)			
Bacteroidetes		2.69	2.76****	0.35***	0.27**	0.39**	-0.39**	0.55
		(0.81)	(0.09)	(0.11)	(0.12)	(0.16)	(0.15)	
	Sphingobacteria	2.49	2.52****	0.30**	0.28**	0.36**	-0.35**	0.51
		(0.79)	(0.09)	(0.11)	(0.11)	(0.16)	(0.15)	
Chloroflexi		1.93	1.89****	0.04	0.09*	-0.23****		0.57
		(0.27)	(0.04)	(0.04)	(0.05)	(0.04)		
Firmicutes		2.02	1.99****	0.04	0.46**		0.43**	0.41
		(0.63)	(0.16)	(0.18)	(0.18)		(0.17)	
	Bacilli	1.94	1.85****	0.03	0.36**	0.42**		0.40
		(0.62)	(0.15)	(0.16)	(0.16)	(0.15)		
Gemmatimonadetes		5.27	5.39****	0.17		0.27	-0.47*	0.13
		(0.47)	(0.15)	(0.16)		(0.24)	(0.25)	
	Gemmatimonadetes	4.60	4.73****	0.07	-0.28	0.37	-0.51**	0.21
		(0.33)	(0.14)	(0.16)	(0.18)	(0.25)	(0.23)	
Nitrospirae		1.45	1.42****	0.11*	0.45****	-0.15	0.13	0.74
		(0.32)	(0.05)	(0.06)	(0.06)	(0.09)	(0.08)	
Planctomycetes		2.10	1.82****	-0.04	0.10	-0.20***		0.31
		(0.45)	(0.06)	(0.06)	(0.07)	(0.06)		
	Planctomycetia	0.96	0.86****	0.00		-0.11***		0.36
		(0.27)	(0.03)	(0.03)		(0.03)		
Proteobacteria		37.67	38.77****	-0.03	-1.05	2.53**	-1.72*	0.14
		(2.69)	(0.59)	(0.68)	(0.73)	(1.03)	(0.96)	
	Alpha	8.79	9.17****	0.34	0.68**	1.16***	-0.52	0.66
		(1.37)	(0.19)	(0.22)	(0.24)	(0.33)	(0.31)	
	Beta	15.74	16.75****	-0.40	-1.75***	1.32	-0.78	0.24
		(0.88)	(0.46)	(0.54)	(0.58)	(0.81)	(0.76)	
	Delta	10.26	9.30****	-0.60**	0.03	-0.48*		0.28
		(1.18)	(0.23)	(0.26)	(0.28)	(0.26)		
	Gamma	1.63	2.34****	0.63***	0.02	0.43	-0.30	0.50
		(0.17)	(0.14)	(0.17)	(0.18)	(0.25)	(0.24)	
Verrucomicrobia		7.70	6.71****	-0.38	-0.86*	-0.56		0.26
		(2.02)	(0.35)	(0.40)	(0.43)	(0.39)		
	Pedosphaerae	1.69	1.62****	0.00		-0.15*		0.06
		(0.46)	(0.08)	(0.09)		(0.09)		
	Spartobacteria	5.70	4.78****	-0.34	-0.80*	-0.86	0.60	0.27
		(2.06)	(0.32)	(0.38)	(0.40)	(0.57)	(0.53)	

Standard error reported in parantheses for model coefficient; standard deviation reported for relative abundances

* p < 0.1; ** p < 0.05; *** p < 0.01; **** p < 0.001

Table 3. Drivers of relative abundance for the most abundant microbial groups on actively managed farms. Relative abundances are expressed as percentages. Model coefficients were selected to maximize adjusted R^2. Model coefficients are standardized and thus represent the effect of a one standard-deviation change in the predictor variable. R^2 for mixed effects models represents the amount of variance explained only by the fixed effects.

Phylum	Class	Order	Mean relative abundance on low fertilizer farms (%)	Intercept	High Fertilizer	High Fertilizer + Agroforestry	pH	Carbon (%)	Nitrogen (%)	Texture	Adj. R^2
Acidobacteria			14.20	14.65****	-3.19**	-1.88	-3.11**				0.21
			(0.03)	(0.91)	(1.35)	(1.45)	(1.22)				
	Acidobacteria		5.02	5.44****	-1.40	0.15	-2.86***				0.30
			(2.45)	(0.60)	(0.88)	(0.95)	(0.80)				
	Acidobacteria 6		3.16	3.16****	-0.44	-0.55					-0.03
			(0.57)	(0.31)	(0.41)	(0.48)					
	Chloracidobacteria		1.59	1.59****	-0.49**	-0.68**					0.20
			(0.61)	(0.17)	(0.20)	(0.24)					
	Solibacteres		2.34	2.34****	-0.40	-0.42					0.01
			(0.76)	(0.21)	(0.29)	(0.33)					
Actinobacteria			15.51	15.77****	3.42	5.11*		5.11**			0.22
			(0.02)	(1.62)	(2.38)	(2.67)		(2.26)			
	Actinobacteria		8.64	8.90****	4.77*	5.87**		5.11**			0.28
			(2.21)	(1.65)	(2.29)	(2.59)		(2.26)			
	Thermoleophilia		6.00	6.00****	-1.18**	-0.80					0.11
			(1.08)	(0.38)	(0.50)	(0.58)					
Bacteroidetes			4.37	4.13***	1.64	2.01	3.00	-2.92		2.78	0.14
			(0.02)	(1.27)	(1.96)	(2.01)	(1.78)	(1.77)		(1.70)	
	Sphingobacteria		3.98	4.26***	-0.33	2.94				2.25	0.04
			(2.32)	(1.29)	(1.77)	(1.97)				(1.70)	
Chloroflexi			2.70	2.70****	-0.39	-0.39					-0.06
			(0.01)	(0.33)	(0.46)	(0.53)					
Firmicutes			2.20	1.81**	1.11	2.98**	4.25****			1.80*	0.46
			(0.03)	(0.76)	(1.13)	(1.20)	(1.05)			(1.02)	
	Bacilli		2.08	1.70**	0.95	2.88**	4.16****			1.79*	0.47
			(2.67)	(0.74)	(1.10)	(1.17)	(1.02)			(0.99)	
Gemmatimonadetes			4.97	4.97****	-0.82*	-1.58***					0.08
			(0.02)	(0.49)	(0.42)	(0.50)					
	Gemmatimonadetes		4.60	4.60****	-0.85*	-1.73***					0.10
			(1.87)	(0.51)	(0.45)	(0.53)					
Nitrospirae			1.21	1.17****	-0.10	-0.24				-0.35**	0.23
			(0.00)	(0.10)	(0.14)	(0.16)				(0.13)	
Planctomycetes			1.82	1.82****	-0.10	-0.09					-0.09
			(0.00)	(0.15)	(0.20)	(0.23)					
	Planctomycetia		1.02	1.02****	-0.07	0.05					-0.08
			(0.29)	(0.09)	(0.13)	(0.15)					
Proteobacteria			37.70	37.50****	0.51	-4.13**		-4.09**			0.20
			(0.05)	(1.18)	(1.73)	(1.95)		(1.64)			
	Alpha		10.76	10.25****	-0.42	-0.49	2.60*	-2.51			0.09
			(4.97)	(1.10)	(1.59)	(1.65)	(1.47)	(1.50)			
		Rhizobiales	3.68	3.68****	-0.50	0.44					0.11
			(1.15)	(0.27)	(0.36)	(0.42)					
	Beta		14.60	14.41****	2.52*	-2.71*			-3.42**		0.20
			(4.43)	(0.98)	(1.29)	(1.46)			(1.34)		
	Delta		8.50	8.50****	-0.63	-2.73**					0.12
			(2.16)	(0.77)	(0.84)	(0.99)					
	Gamma		2.38	2.38****	0.57	0.69*					0.09
			(0.49)	(0.24)	(0.32)	(0.37)					
Verrucomicrobia			6.97	6.97****	-0.66	-2.38****					0.20
			(0.01)	(0.53)	(0.43)	(0.52)					
	Pedosphaerae		1.32	1.32****	-0.09	-0.19					-0.08
			(0.50)	(0.17)	(0.24)	(0.27)					
	Spartobacteria		5.34	5.34****	-0.68	-2.18***					0.19
			(1.42)	(0.50)	(0.44)	(0.52)					

Standard error reported in parantheses for model coefficient; standard deviation reported for relative abundances

* p < 0.1; ** p < 0.05; *** p < 0.01; **** p < 0.001

Table 4. Soil properties and crop yield for experimental plots and actively managed farms to a depth of 20 cm. Texture and bulk density on experimental plots were only taken on control plots. Mean values are reported with standard deviation in parentheses.

	pH	**Clay** (%)	**Sand** (%)	**Silt** (%)	**Bulk density** ($g\ cm^{-3}$)	**Microbially available C** ($\mu g\ CO_2\text{-}C\ g\ soil^{-1}$)	**Active microbial biomass** ($\mu g\ CO_2\text{-}C\ g\ soil^{-1}\ h^{-1}$)	**C** (%)	**N** (%)	**P** (ppm)	**Soil moist.** (% vol.)	**Soil temp.** (°C)	**Water holding capacity** ($g\ H_2O\ g\ soil^{-1}$)	**Crop yield** ($t\ ha^{-1}$)
Experimental Plots														
0	5.75	34.80	51.60	13.45	1.09	150.73	1.32	2.13	0.21	6.25	34.31	16.11	29.23	1.52
	(0.32)	(5.31)	(4.87)	(2.20)	(0.05)	(18.91)	(0.23)	(0.21)	(0.02)	(2.87)	(0.92)	(0.43)	(0.37)	(0.23)
50	5.54					218.86	1.02	2.12	0.22	8.00	34.25	15.44	29.19	1.95
	(0.33)					(131.60)	(0.23)	(0.20)	(0.01)	(2.71)	(1.56)	(0.59)	(0.89)	(0.98)
75	5.73					217.41	1.28	2.17	0.22	7.00	35.92	15.53	30.05	1.36
	(0.31)					(44.02)	(0.52)	(0.15)	(0.01)	(2.16)	(1.01)	(0.54)	(0.92)	(0.26)
100	5.53					244.85	1.03	2.14	0.22	7.00	33.69	15.67	27.51	1.95
	(0.16)					(69.42)	(0.25)	(0.10)	(0.02)	(1.83)	(1.30)	(0.61)	(1.52)	(0.68)
200	5.39					171.21	1.02	2.13	0.21	7.00	33.58	15.28	28.58	2.17
	(0.25)					(20.98)	(0.25)	(0.06)	(0.01)	(2.45)	(1.27)	(0.19)	(0.72)	(0.38)
Active Farms														
Low Fertilizer	5.41	31.74	53.76	14.40	1.20	215.75	1.02	1.83	0.20	16.63	24.78	19.01	29.91	0.86
	(0.35)	(6.34)	(5.64)	(7.61)	(0.14)	(46.53)	(0.55)	(0.20)	(0.03)	(9.15)	(3.35)	(1.15)	(2.08)	(0.40)
High Fertilizer	5.05	34.15	56.00	9.77	1.16	259.6	0.86	1.95	0.22	19.13	28.45	18.07	28.78	2.67
	(0.34)	(6.57)	(3.13)	(5.91)	(0.11)	(25.67)	(0.31)	(0.16)	(0.03)	(10.30)	(4.56)	(1.46)	(2.08)	(1.22)
High + Agroforestry	5.47	30.86	58.58	10.46	1.09	425.74	0.72	1.72	0.18	7.00	24.63	19.20	28.38	3.25
	(0.72)	(4.96)	(2.06)	(4.67)	(0.14)	(135.66)	(0.27)	(0.27)	(0.02)	(2.55)	(3.02)	(0.63)	(2.47)	(1.02)

Table 5. Regression model results of taxonomic diversity from experimental plots (1, 2) and managed farms (3, 4). Models using piecewise regression show significant effects of fertilization on bacterial diversity. Spatially explicit generalized least squares and mixed effects modeling show no impact of fertilization on diversity on managed farms (3, 4). Adjusted R^2 is reported as a measure of goodness-of-fit for piecewise models; AIC score is given for mixed effects models. The breakpoint of the piecewise models is the level of fertilizer addition (kg N ha^{-1}) to both sides of which a separate model was fitted.

	Experimental Plots			**Active Farms**	
	Piecewise regression			*Linear mixed effects*	*Spatially explicit generalized least squares*
	Faith's PD	**Shannon**		**Faith's PD**	**Shannon**
	(1)	(2)		(3)	(4)
Breakpoint	79.6	50.00	*Intercept*	360.78****	11.04****
	(36.63)	(29.57)		(7.52)	(1.11)
Nitrogen			*High Fertilizer*	-7.70	-0.20
Intercept	274.90	9.19		(12.01)	(0.21)
(<Breakpoint)			*High + Agroforestry*	-9.24	-0.22
Slope	-0.19*	-0.01**		(11.18)	(0.20)
(<Breakpoint)	(0.09)	(0.00)	*Soil pH*	-7.48	-0.29*
Intercept	261.10	8.87		(9.44)	(0.17)
(>=Breakpoint)			*Percent C*	13.64	0.68
Slope	-0.01	-0.00		(13.31)	(0.54)
(>=Breakpoint)	(0.06)	(0.00)	*Percent N*	-1.81	-3.66
Soil pH	-3.13	0.33		(12.07)	(3.14)
	(8.52)	(0.19)	*Texture*	-11.94	
Percent C	25.61	0.39	(Sand/Silt) + Clay	(9.14)	
	(21.66)	(0.55)			
Percent N	-302.07	-7.67			
	(200.00)	(4.42)			
D.F.	13	13		21	21
Adj. R2	0.30	0.35	AIC	149.05	21.99

Standard error reported in parantheses

* p < 0.1; ** p < 0.05; *** p < 0.01; **** p < 0.001

Table 6. Results from models of functional gene abundances on experimental plots and actively managed farms. Results for other genes are reported in Table 7. Standard error is reported in parentheses. * $p < 0.1$; ** $p < 0.05$; *** $p < 0.01$; **** $p < 0.001$

Gene categories	Intercept	Fertilization (kg N ha^{-1})	High Fertilizer	High Fertilizer + Agroforestry	pH	Carbon (%)	Nitrogen (%)	Texture	Adj. R^2
Managed farms									
C cycling	1130.99****		75.35	245.03****	-28.23	82.52	36.53	29.34	0.34
	(34.49)		(55.08)	(56.46)	(48.93)	(56.71)	(58.19)	(45.89)	
C degradation	915.91****		40.80	167.13***	-18.67	62.48	22.38	22.56	0.24
	(27.07)		(42.55)	(43.71)	(38.21)	(44.46)	(45.61)	(36.17)	
C fixation	179.16****		32.45***	68.86****	-5.82	16.53	11.24	5.96	0.60
	(6.62)		(10.57)	(10.84)	(9.39)	(10.88)	(11.17)	(8.81)	
N and P cycling									
Assimilatory N reduction	40.04****		11.13**	20.66****	-3.76	7.50*			0.50
	(2.67)		(4.17)	(4.31)	(3.67)	(3.78)			
Denitrification	245.12****		22.51	71.08****	-5.26	22.72	6.90	12.35	0.37
	(9.53)		(15.22)	(15.60)	(13.52)	(15.67)	(16.08)	(12.68)	
Dissimilatory N reduction	55.39****		-0.18	5.01***		3.38**		4.61***	0.44
	(1.02)		(1.53)	(1.65)		(1.40)		(1.32)	
N fixation	190.33****		-26.04****	-9.53*		3.35	10.02*	4.85	0.46
	(3.23)		(4.85)	(5.25)		(5.26)	(5.54)	(4.24)	
Phosphorus utilization	147.80****		17.27**	32.06***	-8.63	13.77	5.00		0.42
	(4.94)		(7.86)	(8.11)	(6.78)	(8.16)	(8.36)		
Experimental plots									
C cycling	1717.55****	95.16			305.58*		-239.07		0.15
	(66.93)	(74.95)			(151.78)		(139.58)		
C degradation	1345.31****	71.60			235.52*		-184.12		0.16
	(50.61)	56.68			(114.78)		(105.56)		
C fixation	307.58****	18.97			58.13*		-45.32		0.14
	(13.24)	(14.82)			(30.02)		(27.61)		
N and P cycling									
Assimilatory N reduction	70.86****	4.69			13.07*		-11.47*		0.18
	(2.94)	(3.29)			(6.66)		(6.13)		
Denitrification	407.55****	23.85			89.45**		-72.04*		0.22
	(17.09)	(19.14)			(38.76)		(35.65)		
Dissimilatory N reduction	70.82****	5.36			18.28**	-8.40			0.09
	(3.51)	(3.98)			(8.51)	(7.80)			
N fixation	204.40****	-1.10			28.64*				0.13
	(6.64)	(7.43)			(14.86)				
Phosphorus utilization	229.27****	12.10			38.65*		-31.92*		0.15
	(8.65)	(9.69)			(19.62)		(18.04)		

Table 7. Results from models of key functional gene abundances under resource addition on experimental plots and actively managed farms. C cycling and denitrification genes are significantly higher on farms with resource addition. N limitation and fixation genes are significantly lower with resource addition. Fertilizer addition on experimental plots does not impact functional genes. Certain genes were selected that are related to certain parts of C, N, and P cycling; results for other select genes are reported in Table 3. The variables in each model were selected to optimize adjusted R^2. R^2 for mixed effects models represents the amount of variance explained only by the fixed effects.

Gene categories	Intercept	Fertilization (kg N ha-1)	High Fertilizer	High Fertilizer + Agroforestry	pH	Carbon (%)	Nitrogen (%)	Texture	Adj. R2
Managed farms									
N limitation	139.09****		-16.25**	-9.44	9.30	3.43	11.32		0.21
	(4.75)		(6.56)	(6.84)	(6.12)	(7.47)	(7.60)		
Nitrification	6.68****		-0.86*	0.11				0.47	0.05
	(0.34)		(0.44)	(0.50)				(0.45)	
Cellulose degradation	67.81****		12.08***	21.75****		4.52	0.55		0.43
	(2.87)		(3.49)	(3.97)		(4.38)	(4.53)		
Chitin degradation	144.71****		10.47	33.65***	-5.68	10.02	4.30		0.35
	(4.75)		(7.38)	(7.64)	(6.49)	(7.83)	(8.01)		
Lignin degradation	93.08****		19.27**	35.79****	-8.00	7.99	5.21		0.50
	(4.38)		(6.97)	(7.19)	(6.01)	(7.23)	(7.41)		
Hemicellulose degradation	139.14****		2.16	19.70***		12.52**	2.95		0.33
	(3.42)		(4.97)	(5.57)		(5.60)	(5.90)		
Pectin degradation	1.63****		1.75***	2.47****		0.66			0.44
	(0.37)		(0.54)	(0.61)		(0.52)			
Starch degradation	295.27****		-20.30*	2.07	5.47	8.16	5.97	9.77	0.01
	(5.94)		(9.49)	(9.73)	(8.43)	(9.77)	(10.03)	(7.91)	
CH4 oxidation	21.57****		-1.19	1.62			1.67		0.06
	(0.70)		(1.05)	(1.17)			(1.03)		
CH4 production	7.81****		2.83*	5.60***		3.15**			0.39
	(0.94)		(1.38)	(1.54)		(1.31)			
Ammonification	110.13****		7.32	20.92****		5.97	6.12		0.39
	(3.02)		(4.54)	(5.06)		(4.95)	(5.24)		
Phosphate limitation	625.00****		-31.04	-26.20	20.09	1.69	25.98	15.90	-0.08
	(14.44)		(14.44)	(19.36)	(17.79)	(21.37)	(21.81)	(18.12)	
Experimental plots									
N limitation	220.54****	5.20			35.66	-84.62**	45.85		0.11
	(10.21)	(11.87)			(25.42)	(35.79)	(33.60)		
Nitrification	7.23****	0.18							-0.02
	(0.20)	(0.21)							
Cellulose degradation	117.33****	7.40			22.37*		-17.72		0.11
	(5.48)	(6.14)			(12.43)		(11.43)		
Chitin degradation	218.38****	12.83			39.25*		-29.73		0.17
	(8.35)	(9.35)			(18.94)		(17.42)		
Lignin degradation	160.14****	15.64**			17.58		-24.48*		0.26
	(5.87)	(6.58)			(13.32)		(12.25)		
Hemicellulose degradation	204.53****	9.11			32.50*		-29.59*		0.14
	(7.74)	(8.66)			(17.54)		(16.14)		
Pectin degradation	6.41****	1.05**					-2.31**		0.40
	(0.42)	(0.43)					(0.85)		
Starch degradation	356.62****	12.54			73.93**	-35.71			0.09
	(13.86)	(15.73)			(33.61)	(30.79)			
CH4 oxidation	33.73****	3.21**			5.67*	-4.80			0.16
	(1.32)	(1.50)			(3.20)	(2.93)			
CH4 production	20.62****	2.13			6.00	-3.68			0.03
	(1.45)	(1.65)			(3.52)	(3.23)			
Ammonification	164.07****	9.72			37.34**	-25.10*			0.20
	(5.93)	(6.73)			(14.38)	(13.18)			
Phosphate limitation	749.02****	10.40			67.99	-234.88**	139.85		0.14
	(26.23)	(30.51)			(65.34)	(91.97)	(86.34)		

Standard error reported in parantheses for model coefficient

* p < 0.1; ** p < 0.05; *** p < 0.01; **** p < 0.001

2.6.2 Figures

Figure 1. Map of the study area. Kenya is shown in red. The study area is located in Nyanza province in Western Kenya between the city of Kisumu and the border with Uganda. Dots represent individual farms from the actively managed farms treatments and are labeled according to management approach. The point labeled 'Experimental Plots' is the project site for the Millennium Villages Project where experimental fertilizer plots are maintained. Experimental plots are located at 0°06'04.88 N and 34°30'40.12 E at an elevation of 1450 m.

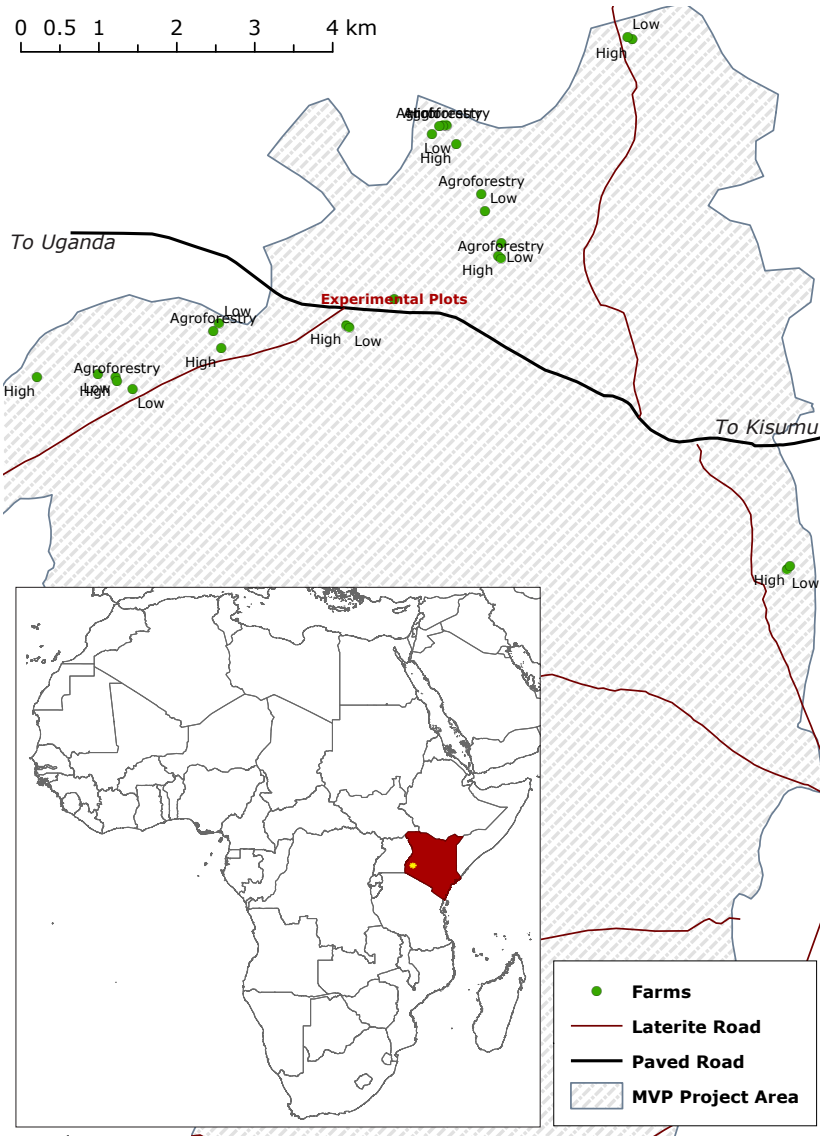


Figure 2. Diversity of OTUs decreases with fertilization. Mean regression line is white, individual bootstrapped regressions are darker. Darker areas represent higher confidence. Data points (b, d) are jiggered for visibility.

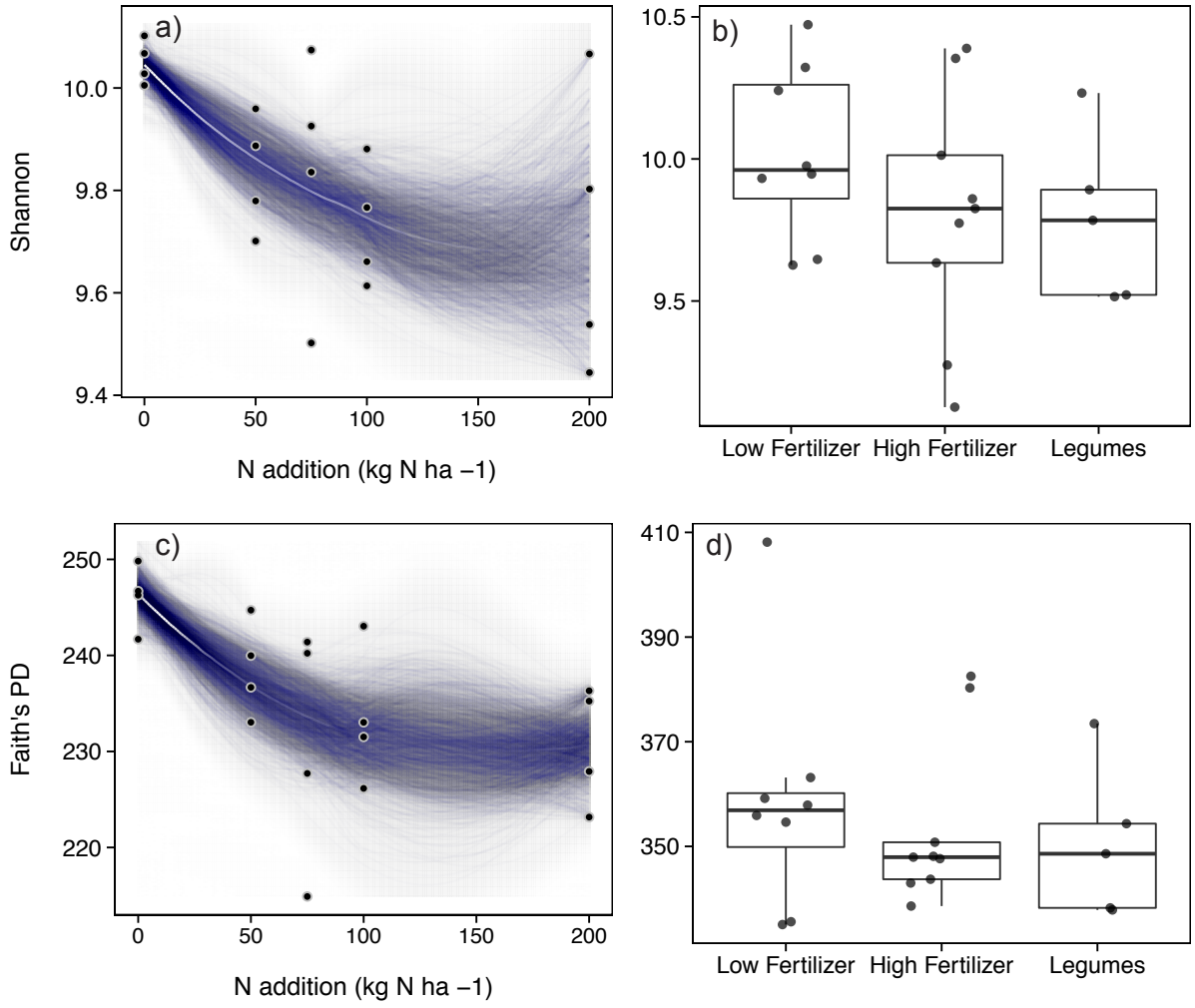


Figure 3. Microbial communities shift towards copiotrophic dominance with fertilization. Mean regression line is white, individual bootstrapped regressions are darker. Darker areas represent higher confidence. Black line shows the slope of the full model. Data points (b, d) are jiggered for visibility.

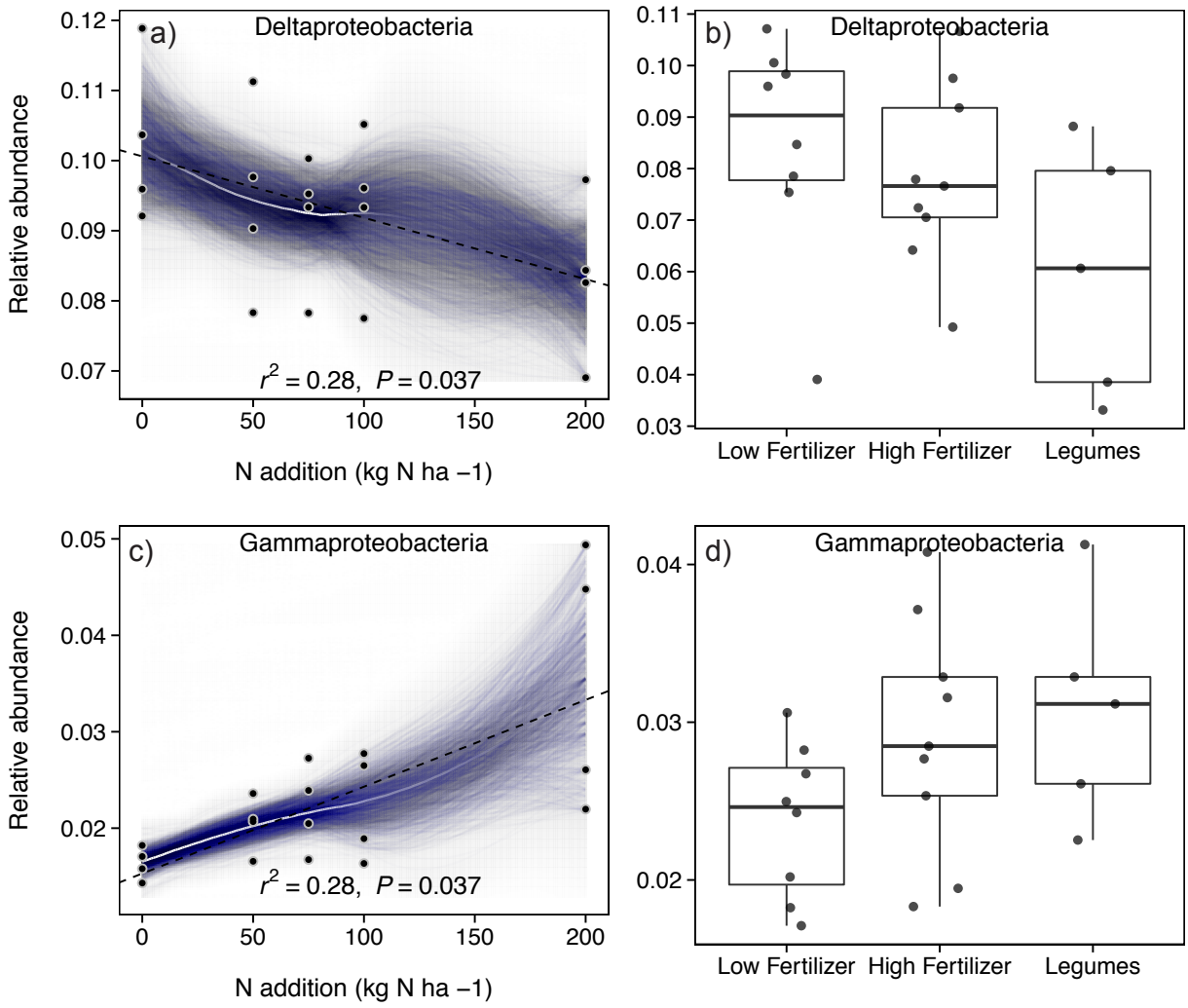


Figure 4. Variability in taxonomic diversity and relative abundance of key microbial taxa increases from 0 to 200 kg N ha^{-1}. Variability is calculated as the coefficient of variation (CV) of Shannon diversity (A), Faith's PD (C), and the relative abundance of *Gammaproteobacteria* (B), which represents copiotrophic taxa, and *Deltaproteobacteria* (D), which represents oligotrophic taxa. CV = mean/standard deviation.

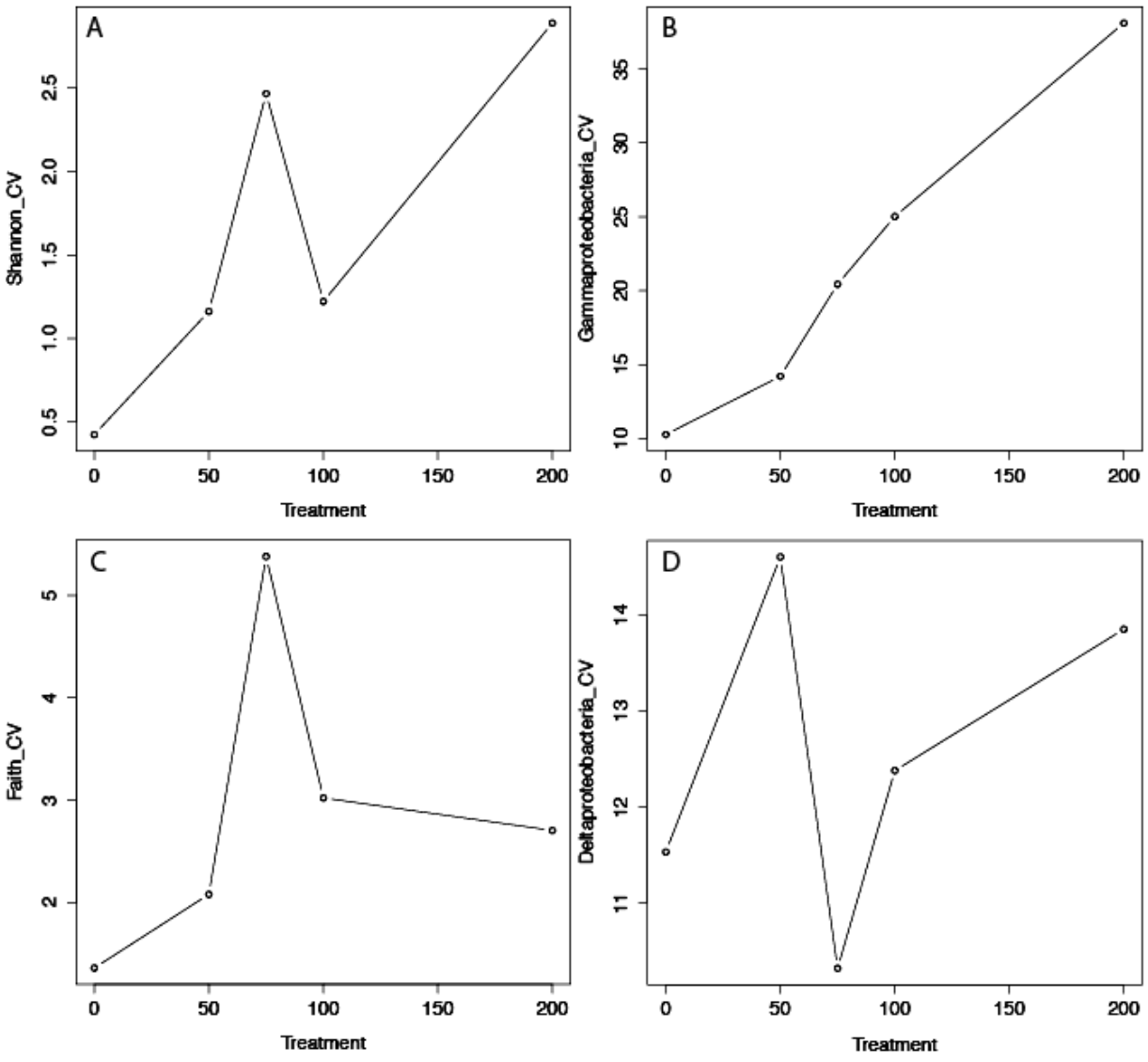
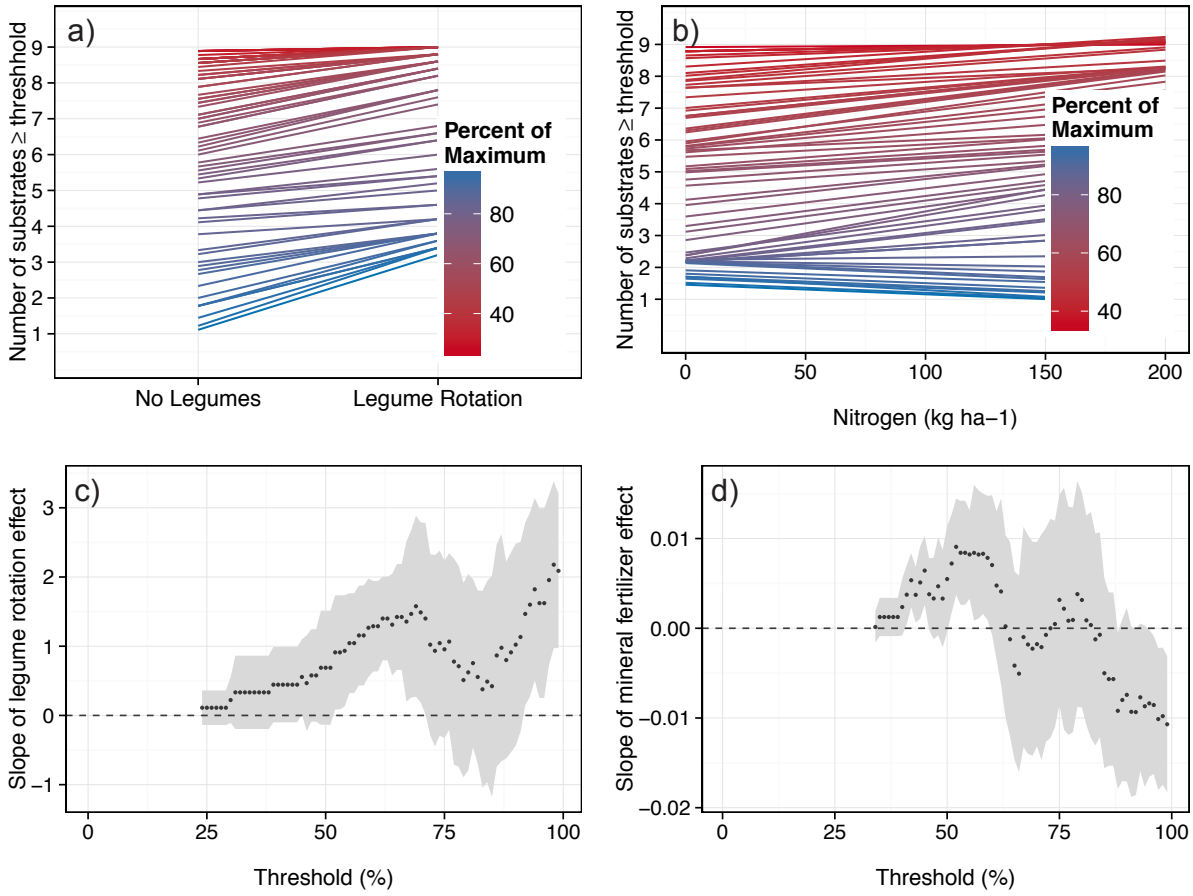


Figure 5. Catabolic capacity is highest under legume rotation at high levels of functioning. Capacity for a farm or plot is the number of substrates whose observed CO_2-efflux rate is greater than or equal to a threshold of the maximum value for each substrate. Individual regressions were run for each threshold (a, b) and coefficients plotted across all thresholds (c, d).



CHAPTER 3: FARM MANAGEMENT, NOT SOIL MICROBIAL DIVERSITY, CONTROLS NUTRIENT LOSS FROM SMALLHOLDER TROPICAL AGRICULTURE

Published as:

Wood, S.A., Almaraz, M., Bradford, M.A., McGuire, K.L., Naeem, S., Neill, C., Palm, C.A., Tully, K.L., & Zhou, J. (2015) Farm management, not soil microbial diversity, controls nutrient loss from smallholder tropical agriculture. *Frontiers Microbiology* 6, doi:10.3389/fmicb.2015.00090.

3.1 ABSTRACT

Tropical smallholder agriculture supports the livelihoods of over 900 million of the world's poorest people. This form of agriculture is undergoing rapid transformation in nutrient cycling pathways as international development efforts strongly promote greater use of mineral fertilizers to increase crop yields. These changes in nutrient availability may alter the composition of microbial communities with consequences for rates of biogeochemical processes that control nutrient losses to the environment. Ecological theory suggests that altered microbial diversity will strongly influence processes performed by relatively few microbial taxa, such as denitrification and hence nitrogen losses as nitrous oxide, a powerful greenhouse gas. Whether this theory helps predict nutrient losses from agriculture depends on the relative effects of microbial community change and increased nutrient availability on ecosystem processes. We find that mineral and organic nutrient addition to smallholder farms in Kenya alters the taxonomic and functional diversity of soil microbes. However, we find that the direct effects of farm management on both denitrification and carbon mineralization are greater than indirect

effects through changes in the taxonomic and functional diversity of microbial communities. Changes in functional diversity are strongly coupled to changes in specific functional genes involved in denitrification, suggesting that it is the expression, rather than abundance, of key functional genes that can serve as an indicator of ecosystem process rates. Our results thus suggest that widely used broad summary statistics of microbial diversity based on DNA may be inappropriate for linking microbial communities to ecosystem processes in certain applied settings. Our results also raise doubts about the relative control of microbial composition compared to direct effects of management on nutrient losses in applied settings such as tropical agriculture.

3.2 INTRODUCTION

Agricultural management, such as mineral nutrient addition, can lead to marked changes in the taxonomic composition of soil microbial communities (Ramirez et al. 2010, 2012, Fierer et al. 2012, Wood et al. 2015b). The pairing of mineral and organic nutrient addition to agriculture can significantly impact the ability of soil microbial communities to catabolize a range of carbon (C) substrates as well as affect the abundance of microbial functional genes involved in multiple aspects of C, nitrogen (N), and phosphorus (P) cycling (Wood et al. 2015b). Some of the microbially driven processes associated with these changes in functional capacity, such as denitrification and decomposition, determine the retention and loss of nutrients in ecosystems and are thus important to managing agriculture for crop production while minimizing nutrient losses to the environment (Vitousek et al. 2009). There is thus keen interest in whether changes in microbial community composition can directly impact rates of ecosystem processes (e.g. Wessen et al. 2011, Wallenstein and Hall 2011, van der Heijden and Wagg 2012, Philippot et al. 2013, Krause et al. 2014).

Certain ecosystem processes are likely to be more sensitive to changes in microbial community composition than others. Narrow processes are most likely to be affected by changes in community composition because they require a specific physiological pathway and/or are carried out by a phylogenetically clustered group of organisms (Schimel and Schaeffer 2012). Thus, processes can be either physiologically narrow, phylogenetically narrow, or both. In this manuscript we use the term "narrow" to refer to physiologically narrow processes that require specific physiological pathways, regardless of their distribution in the microbial phylogeny. For instance, we refer to denitrification as a narrow process because it requires particular genes that code for enzymes capable of reducing various forms of nitrogen. Because a relatively small proportion of microorganisms carry these genes, changes in community composition that lead to a shift in the relative abundance of denitrifiers—or changes in the abundances of the relevant functional genes—should have significant impacts on rates of denitrification (Pett-Ridge and Firestone 2005, Philippot et al. 2013, Powell et al. 2015). Mineralization of soil C to CO_2, by contrast, is a broad process because the ability to mineralize and respire C substrates is relatively simple and shared by many microbial taxa (Schimel and Schaeffer 2012). We thus expect that carbon mineralization would not respond strongly to changes in the composition of microbial communities.

Whether this framework of broad and narrow processes helps predict nutrient losses from agriculture depends on the relative importance of the multiple potential drivers of ecosystem process rates, including microbial community composition, nutrient availability, and soil and environmental properties. Though several studies have found support for microbial influence on narrow processes, such as denitrification, such studies often focus on identifying whether microbial community composition is related to ecosystem processes, but stop short of

quantifying the relative contribution of the multiple controls on ecosystem processes (e.g. Philippot et al. 2013). Understanding the importance of biodiversity requires assessing the influence of composition relative to other biotic and abiotic controls (Laliberte and Tylianakis 2011, Bradford et al. 2014c, 2014b).

Following theory (Schimel 1995, Schimel and Schaeffer 2012), we hypothesize that changes in microbial diversity will have a stronger effect on denitrification than will the direct effect of nutrient addition—measured as both N addition and the inclusion of seasonal legume rotations (henceforth *agroforestry*) to increase soil C—if changes in diversity correspond with changes in the relative abundance of denitrifying taxa and the abundances of functional genes involved in denitrification. Because C mineralization is a broad process, we expect that nutrient addition will have a stronger effect on process rates than changes in the microbial community.

3.3 Materials and Methods

3.3.1 Site selection

We examine our hypotheses on 24 smallholder farms in western Kenya participating in the Millennium Villages Project (MVP) site in Sauri, Kenya (Figure 1; Wood et al. 2015b). The center of the study area is located at 0°06’04.88 N, 34°30’40.12 E at an elevation of 1450 m. The mean annual temperature and precipitation for the study region are 24°C and 1800 mm, respectively. Annual precipitation is distributed bi-modally with 1120 mm in a long rainy season from March to June and 710 mm in a short rainy season from September to December. The soils are classified as Oxisols and are well drained sandy clay loams (on average 53.75% sand, 12.59% silt, 33.54% clay) with a mean pH of 5.45 and C:N of 11.52 (0-20 cm). The study zone was originally part of the moist broadleaf forest area in eastern and central Africa, but is now a

mixed-maize agricultural system, with most farmers cultivating maize in both the long and short rainy seasons. Some farmers, however, replace the short rain maize crop with a seasonal legume rotation that fixes nitrogen and builds soil organic matter.

The MVP was designed to meet the Millennium Development Goals at the village scale in Sub-Saharan Africa and includes an agricultural component that focuses on increasing crop yields through mineral and organic nutrient addition to redress negative soil nutrient balances (Sanchez et al. 2007). This is primarily achieved by subsidizing mineral fertilizers (primarily diammonium phosphate and urea). Farmers are also trained in seasonal legume rotations to fix nitrogen and build soil organic matter. In Sauri, rotational legume trainings have been promoted since the early 1990s (Kiptot et al. 2007) and fertilizer subsidy programs were active from 2005-2008.

We selected farms to participate in the study based on two years of household surveys. We determined nutrient inputs and outputs for each of these farms through a combination of interviews, on-farm crop harvests, and biomass estimations. Farms were classified into three categories: *low fertilizer*, *high fertilizer*, and *high fertilizer + agroforestry* (specifically, seasonal legume rotations). Low fertilizer farms have applied less than 10 kg mineral N ha^{-1} y^{-1} since 2005; high fertilizer farms have applied at least 60 kg N ha^{-1} y^{-1} over the same time period. High fertilizer + agroforestry farms (henceforth *agroforestry*) apply amounts of mineral N comparable to *high fertilizer* farms, but also use agroforestry techniques to build soil organic matter. These agroforestry techniques replace short-rain maize crops with fast-growing leguminous tree, shrub, or herbaceous species that are planted from seed and cut each year for organic inputs to crop fields. These techniques are referred to generally as agroforestry, though agroforestry is a general

term that captures different practices not studied here (e.g., wind breaks, live fencing, etc.). Our results, therefore, apply to agroforestry strategies that seasonally incorporate legume rotations.

We estimated the amount of N added to farms with farmer-reported data on the quantity of N added through mineral and organic sources (diammonium phosphate, urea, biological N_2-fixation, and manure). For agroforestry farms, we also estimated the amount of N added through N_2-fixation based on both literature-reported values and field-reported biomass estimates. To estimate the amount of N added through N_2-fixation we collected data on legume species planted, original planting density, thinning practices, wood harvesting, and legume management. We used plant density to estimate the amount of aboveground biomass N for each species present and then used literature data on the percent of total N derived from biological N_2-fixation for each species to calculate the amount of N derived from fixation (Gathumbi et al. 2002b, 2002a, Ojiem et al. 2007). Because farmers tend to remove woody stems but incorporate fresh leaves, we removed the amount of N stored in woody biomass from this value to estimate the net N contribution from the legume species to the farm fields. We conservatively estimate that N_2-fixation contributed between 30 to 50 kg N ha^{-1} $year^{-1}$ during the short rain fallow (Gathumbi et al. 2002a), up to 30 kg of which may be due to the presence of *Mucuna pruriens*, an annual climbing legume (Ojiem et al. 2007). Planting densities, however, can vary widely from year-to-year with low-density years being as low as an order of magnitude less than those assumed in this estimate. Thus, depending on the year, actual fixation rates may be as low as 5 to 30 kg N ha^{-1} short rainy $season^{-1}$. We use the term 'nutrient addition' to refer to both N addition on low- and high-fertilizer and agroforestry farms as well as C addition through agroforestry. The final farms included in the study are distributed across the Sauri village cluster, but are clustered by treatment (Figure 1) on similar underlying soils.

3.3.2 Sample collection and measurement

Soil sampling was conducted in June 2012, in the middle of the long rains, two weeks after fertilizer application. On the farm fields, we took 15 2-cm diameter soil cores from the top 20 cm of bulk soil. Cores were taken at regular intervals throughout the entire farm field and homogenized and aggregated to a composite sample. At each core location we recorded temperature and volumetric soil moisture content using a soil thermometer and a HydroSense moisture probe (Campbell Scientific, Logan, UT, USA). We sieved soils to 2 mm and stored soil for DNA extraction at -20° C. Soils for DNA extraction were transported to the U.S. within one week of sampling. Subsamples of sieved field soil were stored at 4° C, transported to the U.S. within one week of sampling, and used to determine pH, gravimetric soil moisture, and water holding capacity. Gravimetric soil moisture and water holding capacity (after wetting soils to field capacity) were determined by drying soil at 105°C for 24 h. Soil pH was determined using a benchtop meter of a 1:1 slurry of soil:H2O by volume.

A subsample of sieved soil was air-dried and used to determine total C and total N by combustion with an Elementar Vario Macro CNS analyzer. Total extractable P was assessed by combining a 5-g soil sample with 20 mL of Mehlich I extraction solution and shaking for 5 min followed by inductively coupled plasma spectrometry (Varian Vista MPX Radial ICP-OES). Soil nutrient assays were performed at the Auburn University Soil Testing Laboratory (AL, USA). Sieved, air-dried soil was also used to determine soil texture using the hydrometer method that uses sodium hexametaphosphate to complex the anions that bind to clay and silt particles into aggregates and suspend organic matter in solution. The density of the soil suspension is determined using a hydrometer after the sand particles settle and then after the silt particles settle (Bouyoucos 1962).

Denitrification and C mineralization assays were performed in Kenya on fresh soils at the MVP regional office in Kisumu, Kenya. Denitrification potential was estimated based on N_2O emissions during denitrifying enzyme activity (DEA) assays (Smith and Tiedje 1979). In a 125-mL flask, we combined 20 g of soil with 20 mL of a 1-mM sucrose and 1-mM KNO_3^- solution. We fit each flask with a #5 stopper, which was inserted with a 22G needle capped with a stopcock. We then brought the headspace of the flask to 10% acetylene (C_2H_2) concentration by volume (to inhibit the reduction of N_2O to N_2 via denitrification). At the beginning of the incubation we closed the stopcocks and placed the flasks onto a shaker table at 125 rpm; flasks were only removed from the table for sampling. We sampled the headspace five times: at 30, 150, 210, and 270 min, by removing 30 mL of gas from the headspace and then replacing the volume of headspace that was removed with 30 mL of 10% C_2H_2 room air (fluxes were corrected for N_2O molecules removed at each sampling period). DEA headspace samples were stored in pre-evacuated vials.

Water-amended soil incubations were used to measure CO_2 efflux and, thus, actual C mineralization. These incubations were performed identically to the DEA incubations with three exceptions: (1) 20 mL of deionized water was added to soil in place of the sucrose and KNO_3^- solution; (2) no C_2H_2 was added to the headspace; and (3) headspace samples were taken at only two time points (240 and 360 min). We also sampled room air at the beginning and end of each incubation and included travel standards to accompany samples, in order to correct for any sample loss during transport and storage. DEA and CO_2 headspace samples were transported to the U.S., where we determined N_2O and CO_2 concentrations by gas chromatography using a Shimadzu GC-14 GC with electron capture (for N_2O) and thermal conductivity (for CO_2) detectors at the Cary Institute (Millbrook, NY).

To measure taxonomic diversity, we performed 16S rRNA amplicon sequencing of bacteria and archaea following standard protocols of the Earth Microbiome Project using an Illumina MiSeq instrument (www.earthmicrobiome.org/emp-standard-protocols/; Gilbert et al. 2010, Caporaso et al. 2012). Briefly, we extracted DNA using a MoBio PowerSoil 96-well extraction kit and we amplified the 16S rRNA V4 gene from bacterial and archaeal genomes using the primers 515F (forward) and 806R (reverse) (Caporaso et al. 2012). The 16S rRNA gene is a well-conserved gene in bacteria and thus captures evolutionary relationships among bacterial taxa. Quality filtering was performed by comparing input sequences with Phred scores (Q >= 20). Sequences shorter than 75% of the Phred score were discarded as well as sequences with ambiguous base call characters. All quality filtering and demultiplexing were performed using the split_libraries_fastq.py algorithm in QIIME and its associated default parameters (www.earthmicrobiome.org/emp-standard-protocols/; Caporaso et al. 2010). Sequence reads were were binned into operational taxonomic units (OTUs) at a 97% similarity threshold. OTUs were then compared to GenBank to identify bacterial lineages. A total of 3,462,835 bacterial sequences were generated across all samples, representing 29,195 OTUs. Sequence lengths averaged 150.63±2.93 per sample. Rarefaction was used to compare samples at depth of 40 sequences per sample. We calculated taxonomic diversity as Shannon diversity (H') of all OTUs. We calculated other diversity metrics, such as Faith's PD, and found similar patterns. All data checks and processing were done using QIIME (Caporaso et al. 2010).

To estimate microbial functional diversity, we measured the abundance of key functional genes using GeoChip 4.0 to analyze DNA samples that were extracted following the protocol for taxonomic assessment. GeoChip is a functional gene array of bacteria, archaea, and fungi covering 401 gene categories involved in major biogeochemical and ecological processes, as

previously described (He et al. 2007, Yang et al. 2013, Tu et al. 2014). GeoChip examines the abundance of thousands of functional gene variants simultaneously through a fluorescent procedure. DNA samples were labeled with a fluorescent dye and purified using a QIA quick purification kit (Qiagen, Valencia, CA, USA) following He et al. (2007) and Tu et al. (2014). DNA was then dried in a SpeedVac (ThermoSavant, Milford, MA, USA) and labeled DNA was resuspended in a hybridization solution before hybridization of DNA was carried out on a MAUI hybridization station (BioMicro, Salt Lake City, UT, USA). GeoChip microarrays were scanned by a NimbleGen MS200 scanner (Roche, Madison, WI, USA). Poor quality spots were removed when flagged as one or three by ImaGene (Arrayit, Sunnyvale, CA, USA) or with a signal-to-noise ratio of less than 2.0. Signal-to-noise ratio indicates the amount of luminescence from the sample compared to background noise. Average signal-to-noise ratios are often greater than 50 (He et al. 2007), so 2.0 represents high noise to signal. Processed data were subjected to the following steps: (i) normalize the signal intensity by dividing the signal intensity by the total intensity of the microarray followed by multiplying by a constant; (ii) transform by the natural logarithm; (iii) remove genes detected in only one out of three samples from the same treatment. Signal intensities were quantified and processed using a previously described data analysis procedure (He et al. 2007, Yang et al. 2013). We calculated functional diversity as Shannon diversity (H') of the signal intensity for all of the genes reported from the array. We also analyzed the response of individual denitrification genes to changes in functional diversity. These include genes involved in nitrite reduction (*nirK*, *nirS*), nitrate reduction (*narG*), and nitric oxide reduction (*norB*). GeoChip also includes *nosZ*, which is involved in nitrous oxide reduction, but we do not analyze this gene because it is involved in a later stage of denitrification than represented by the denitrification potential assay.

3.3.3. Data analysis

We used structural equation models to simultaneously estimate each of the pathways among nutrient addition, soil and environmental properties (pH, texture, and moisture), microbial communities, and ecosystem processes while accounting for correlations between multiple response variables (Grace 2006). Structural equation modeling is increasing used in ecology and environmental sciences to assess the relative impacts of multiple variables on each other and a set of response variables (Grace 2006). This technique has been applied to a wide range of issues in ecology and environmental sciences (Byrnes et al. 2011, Flynn et al. 2011, Laliberte and Tylianakis 2011). Relevant to our study, it was used by Colman and Schimel (2013) to determine the drivers of microbial respiration and N mineralization at continental scales.

To test our hypotheses about the relative importance of nutrient addition and microbial composition, we first fitted models including both nutrient addition and microbial diversity variables. Soil pH was the only significant environmental control and was thus the only environmental variable retained in the final models. We then fitted models to optimize goodness-of-fit and do not include variables that do not contribute strongly to model goodness-of-fit. Different models were fitted for each of the two response variables (denitrification potential and C mineralization). For each response variable, constrained (microbial + nutrient addition) and unconstrained models were compared based on change in AIC values. The final, unconstrained model retained nutrient addition and pH, but did not include microbial diversity.

We report standardized path estimates that allow for comparison of the relative magnitude of variables within the same model (Grace and Bollen 2005). For model goodness-of-fit, we report X^2 and root mean square error of approximation (RMSEA). These measures assess

the similarity between the covariance matrix of the observed data and the covariance matrix implied by the specified model. A X^2 P-value greater than 0.05 implies significant overlap between the observed and implied data, and thus adequate model fit. We report Sartorra-Bentler X^2 correction factors to improve estimates based on violations of multivariate normality. Because the X^2 test is based on large sample theory, we also report RMSEA, which is a goodness-of-fit measure weighted by sample size. We use an RMSEA value below 0.1 to represent good model fit because for sample sizes less than 50, the conventional RMSEA cut-off value of 0.05 is overly conservative (Chen et al. 2008). Individual paths were estimated using maximum likelihood and we considered paths to be significant at $P < 0.05$ and marginally significant at $P < 0.10$ (Hurlbert and Lombardi 2009). Insignificant paths were excluded from models unless they significantly improved overall model fit, based on X^2 and RMSEA values as well as assessment of modification indices (Grace 2006). All models were fitted using the *lavaan* package in R (Rosseel 2012).

3.4 RESULTS

We hypothesized that changes in microbial diversity would have a stronger effect on denitrification than would the direct effect of nutrient addition if changes in diversity correspond with changes in the relative abundance of denitrifying taxa and/or the abundance of associated genes involved in denitrification. We also hypothesized that nutrient addition would be a stronger predictor of C mineralization, a broad process, than microbial diversity.

We find that farm management—through N addition and agroforestry—impacts the taxonomic and functional diversity of soil microbial communities. Specifically, taxonomic diversity decreases by 2.40% from low-to-high N addition (Table 1), though this effect is weaker than the effect of pH, which is also associated with lower taxonomic diversity (Figure 2A, B).

We did not find that these changes in taxonomic diversity were coupled with changes in the relative abundance of select groups of denitrifying taxa (Figure 3). Agroforestry was the strongest driver of functional diversity, which increased 1% between high fertilizer and agroforestry farms and 2% between low fertilizer and agroforestry farms (Table 1; Figure 2A, B). We did find that greater functional diversity is significantly related to greater abundances of several genes involved in denitrification: *nirK*, *nirS*, *norB*, and *narG* (Figure 4).

We did not, however, find that changes in taxonomic and functional diversity were related to rates of either denitrification or C mineralization. Instead, ecosystem process rates were most strongly linked to the direct effect of farm management. Denitrification decreased by 21.31% from low-to-high N and increased by 63.93% from low N to agroforestry (Table 1). The path estimate for agroforestry on denitrification (0.63) is three times greater than the coefficient for either taxonomic diversity (-0.24) or functional diversity (-0.18). The agroforestry coefficient is also twice the magnitude of the coefficient for N addition (-0.33). We find support for our hypothesis that C mineralization will be more influenced by nutrient addition than microbial community composition. C mineralization rates were 4.81% lower on high-vs.-low N farms and 22.12% greater under agroforestry (Table 1). The path coefficient for the effect of agroforestry on C mineralization (0.47) is more than twice as great as the coefficient for taxonomic diversity (-0.23) and N addition (0.16) and around five times the effect of functional diversity (-0.08).

3.5 DISCUSSION

Our results reveal that shifts in microbial taxonomic and functional diversity due to farm management are not significantly related to either denitrification or C mineralization on smallholder farms in western Kenya. This finding supports our hypothesis that C mineralization would not be sensitive to changes in microbial communities because it is a broad process that can

be carried out by many microbial taxa. However, we did not find support for our hypothesis that denitrification would be sensitive to community change because it is a narrow process carried out by relatively few taxa.

This unexpected result may be explained by the fact that changes in taxonomic diversity were not coupled with decreases in the relative abundance of denitrifying taxa. Our hypothesis was built on the expectation that diversity would relate to denitrification rates if changes in diversity were paired with changes in the relative abundance of taxa able to carry out denitrification. Because denitrifying taxa are found widely through the microbial phylogeny, it is difficult to identify groups of taxa that are all denitrifiers. However, we found that groups that broadly contain denitrifiers do not change in relative abundance with changes in diversity. This finding may explain why taxonomic diversity was not a significant predictor of denitrification.

We also expected that functional diversity would be a significant control on denitrification if changes in functional diversity were coupled with changes in the abundances of key denitrifying genes. We did find a strong coupling between our functional diversity metric (Shannon diversity of all functional genes from GeoChip) and the abundances of four particular genes key to denitrification: *nirK*, *nirS*, *narG*, and *norB*. Thus, our finding that functional diversity was not significantly related to rates of denitrification was unexpected. However, the finding fits with recent meta-analysis showing that microbial functional gene abundances are rarely strongly correlated with corresponding process rates (Rocca et al. 2014). Our lack of observed relationship between gene abundances and process rates may be explained by the fact that our measure of functional diversity is based on the presence of functional genes using DNA. Because DNA only indicates the presence of a gene, rather than whether that gene is expressed, our measure of functional diversity only represents a coarse picture of microbial functional

capacity. Our results thus suggest that rates of denitrification are more strongly controlled by the expression of functional genes, rather than their overall abundance. This finding suggests that coarse measures of microbial communities based on DNA—whether taxonomic or functional—may be insufficient to understanding the changes in the functional contributions of these communities under certain types of land management (Rocca et al. 2014).

Though understanding when microbial communities should impact ecosystem process rates is well established, we show that actual changes in microbial communities observed in a tropical agroecosystem are not a strong predictor of denitrification rates because changes in microbial communities are relatively minor in magnitude. Our findings, however, do not invalidate the hypothesis that narrow processes are sensitive to community composition and broad processes are not, which has been supported in previous work (e.g. Salles et al. 2009, Schimel and Schaeffer 2012, Philippot et al. 2013, Powell et al. 2015). Instead, our findings raise doubts about the relative importance of microbial community composition compared to direct effects of nutrient addition on nutrient losses in agricultural settings. This is because the magnitude of change in microbial diversity induced by land management was not large enough to significantly impact ecosystem process rates. As a result, the direct effect of farm management (rather than the indirect effect through changes in microbial communities) was the dominant control of both denitrification and C mineralization. Whether changes in microbial community composition translate into changes in rates of ecosystem processes controlled by soil microbes is of great interest in soil ecology (e.g. Torsvik and Øvreås 2002, Philippot and Hallin 2005, van der Heijden et al. 2008), but remains an ongoing debate (Schimel and Schaeffer 2012). Our study is unique, however, in that few studies have connected changes in microbial communities to

ecosystem process rates in a framework that assesses the relative importance of the multiple drivers of these ecosystem processes.

Although we focus on smallholder farms in western Kenya, there is a widespread effort to increase crop yields across sub-Saharan Africa and in tropical smallholder agriculture more generally (Wiggins et al. 2010). Because seventy-five percent of the world's 1.2 billion poorest people are engaged in smallholder, making up 500 million farms of less than 2 ha (Wiggins et al. 2010), our findings may help inform understanding of drivers of nutrient loss in tropical smallholder agriculture due to increased attention from international development organizations.

It is becoming widely recognized that agricultural sustainability requires agricultural practices that maximize multiple ecosystem services while minimizing nutrient losses to the environment (Foley et al. 2011, Bommarco et al. 2013). This is particularly important in tropical ecosystems that are undergoing large-scale modifications of nutrient cycling pathways due to intense efforts by the international development community to increase fertilizer use by tropical smallholder farmers. Further work should focus on understanding how management-induced shifts in microbial communities impact not just potential nutrient loss, but the multiple ecosystem services provided by soil and how such understanding can be integrated into sustainable agricultural strategies.

3.6 TABLES AND FIGURES

3.6.1 Tables

Table 1. Means and standard deviation for variables included in structural equation models among the three categories of nutrient addition: low fertilizer, high fertilizer, and agroforestry. All soil properties are to a depth of 20 cm. Because of unbalanced design statistical comparisons between groups are not valid; instead the effect of Farm Type is represented by the path coefficients of Agroforestry and N Addition in the structural equation models. Further detail on changes in soil properties is presented in Wood et al. (2015b).

Treatment	Denitrification	C mineralization	Taxonomic diversity	Functional diversity	Sand	Silt	Clay	pH
	(ng N g dry soil^{-1} h^{-1})	*(ug C g dry soil^{-1} h^{-1})*	*H'*			%		
Low Fertilizer	0.61 [0.49]	1.04 [0.24]	10.02 [0.31]	9.17 [0.07]	53.76 [5.64]	14.40 [7.61]	31.74 [6.34]	5.41 [0.35]
High Fertilizer	0.48 [0.09]	0.99 [0.41]	9.78 [0.45]	9.27 [0.08]	56.00 [3.13]	9.71 [5.91]	34.15 [6.57]	5.06 [0.37]
Agroforestry	1.00 [0.58]	1.27 [0.13]	9.79 [0.30]	9.33 [0.09]	58.58 [2.06]	10.46 [4.67]	30.86 [4.96]	5.47 [0.72]

Table 2. Model results and goodness of fit statistics for structural equation models. We report robust X^2 statistics for model fit. $P > 0.05$ indicates that estimated models have covariance matrices among variables that are not strongly different from observed values and that the model, therefore, adequately represents the data. Root mean square error of approximation (RMSEA) is a sample-size weighted measure of model fit. Values below 0.1 indicate good model fit.

Denitrification			**C Mineralization**		
	Standardized Estimate	*P*		*Standardized Estimate*	*P*
Denitrification~			C mineralization~		
Agroforestry	0.71	0.00	Agroforestry	0.47	0.00
Functional diversity	-0.32	0.03	Functional diversity	-0.08	0.72
N addition	-0.46	0.02	N addition	-0.01	0.95
Taxonomic diversity	-0.22	0.11	Taxonomic diversity	-0.23	0.35
Taxonomic diversity~			Taxonomic diversity~		
N Addition	-0.37	0.06	N Addition	-0.31	0.18
pH	-0.43	0.00	pH	-0.40	0.01
Functional diversity~			Functional diversity~		
Agroforestry	0.48	0.02	Agroforestry	0.48	0.03
Structural Equation Model Metrics			**Structural Equation Model Metrics**		
n	21		*n*	21	
df	5		*df*	5	
χ^2	1.4		χ^2	2.62	
$P_{\chi 2}$	0.93		$P_{\chi 2}$	0.76	
RMSEA	0.00		RMSEA	0.00	
P_{RMSEA}	0.94		P_{RMSEA}	0.75	

3.7.2 Figures

Figure 1. Maize production in western Kenya mainly occurs on smallholder farms of around 1 hectare (A). Map (B) shows the study farms and their distribution across the Millennium Villages Project site area in western Kenya. Farm types are coded by color.

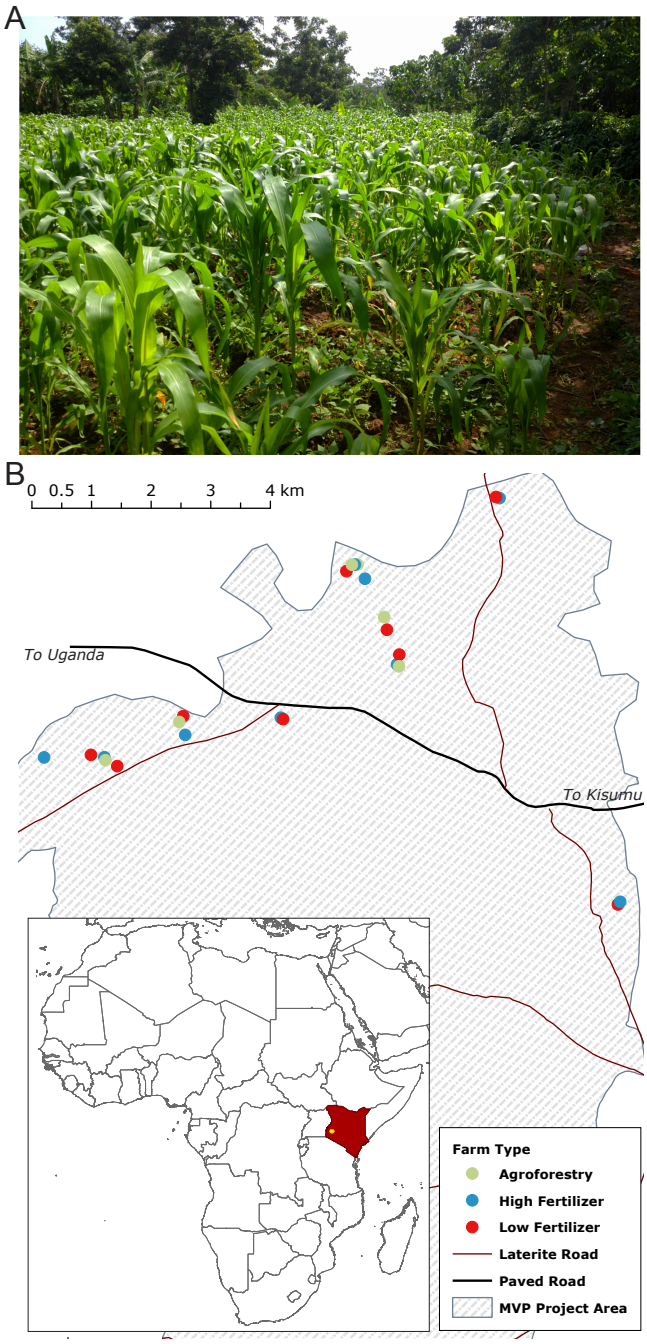


Figure 2. Path diagrams for structural equation models of the relationship between farm management, microbial diversity, and (A) denitrification enzyme activity or (B) carbon mineralization. Models (A, B) show the relative effect of management and microbial diversity. Solid paths are statistically significant at $p < 0.10$. Dashed paths are insignificant, but were included for hypothesis testing or overall model fit. Line color represents effect direction (light green = positive, deep red = negative). Path widths are proportional to standardized regression coefficients, which are shown next to each path. Results and model statistics are in Table 2.

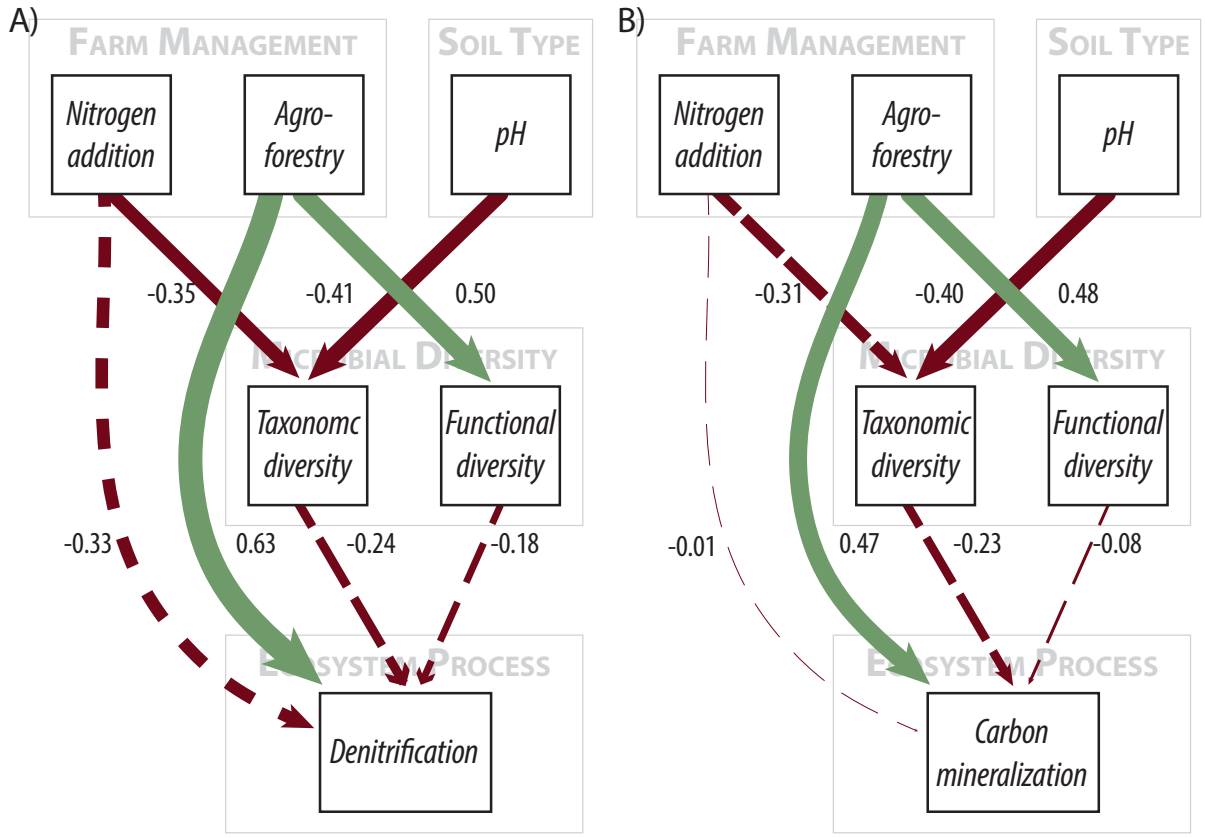


Figure 3. Taxonomic diversity is not related to changes in the relative abundances of select denitrifying taxa. These groups do not represent all categories of denitrifying taxa and not all taxa within these categories are able to carry out denitrification. These groups were selected because they broadly represent evolutionary lineages that are capable of denitrification and had relatively high relative abundances in our samples.

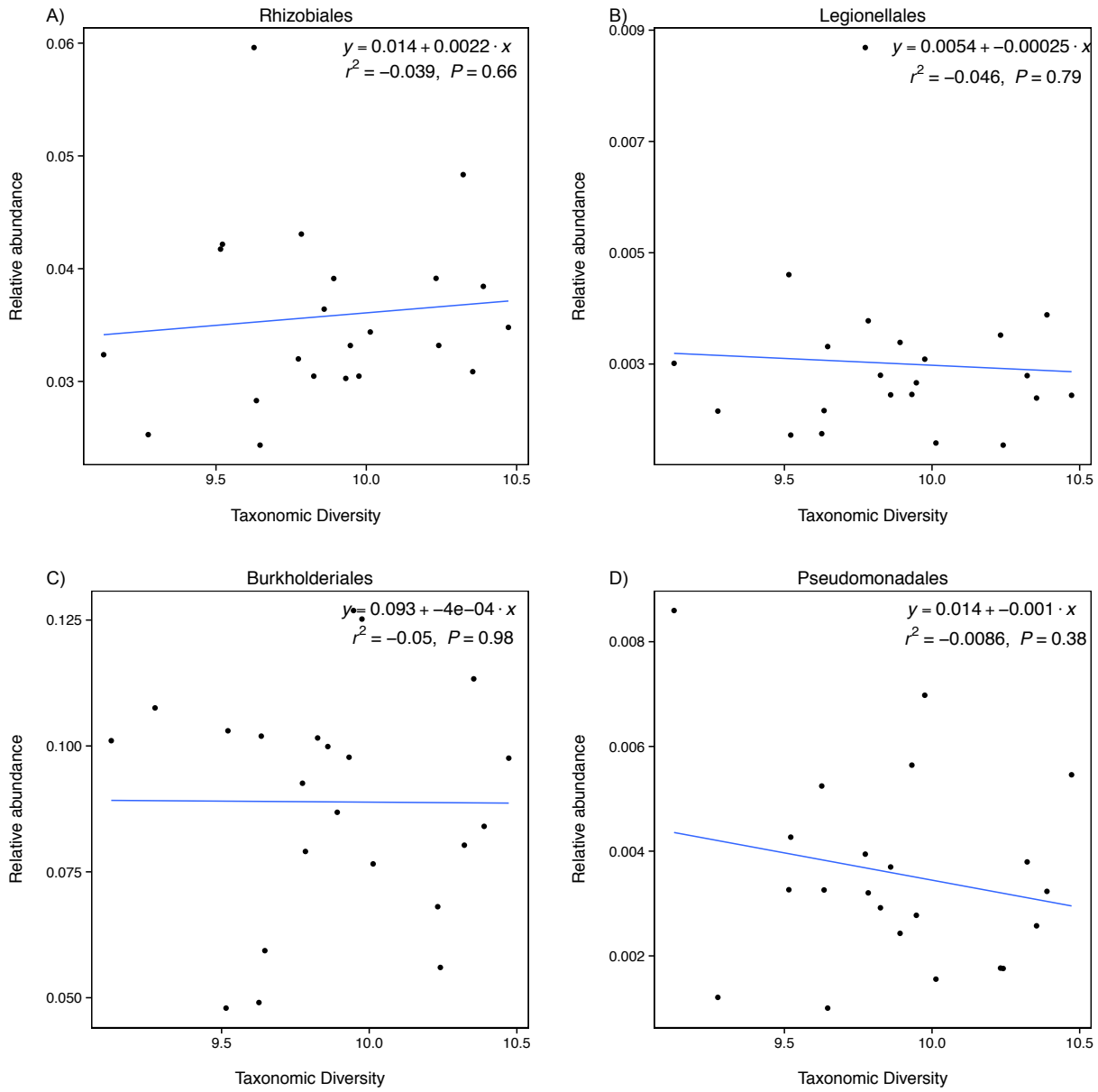
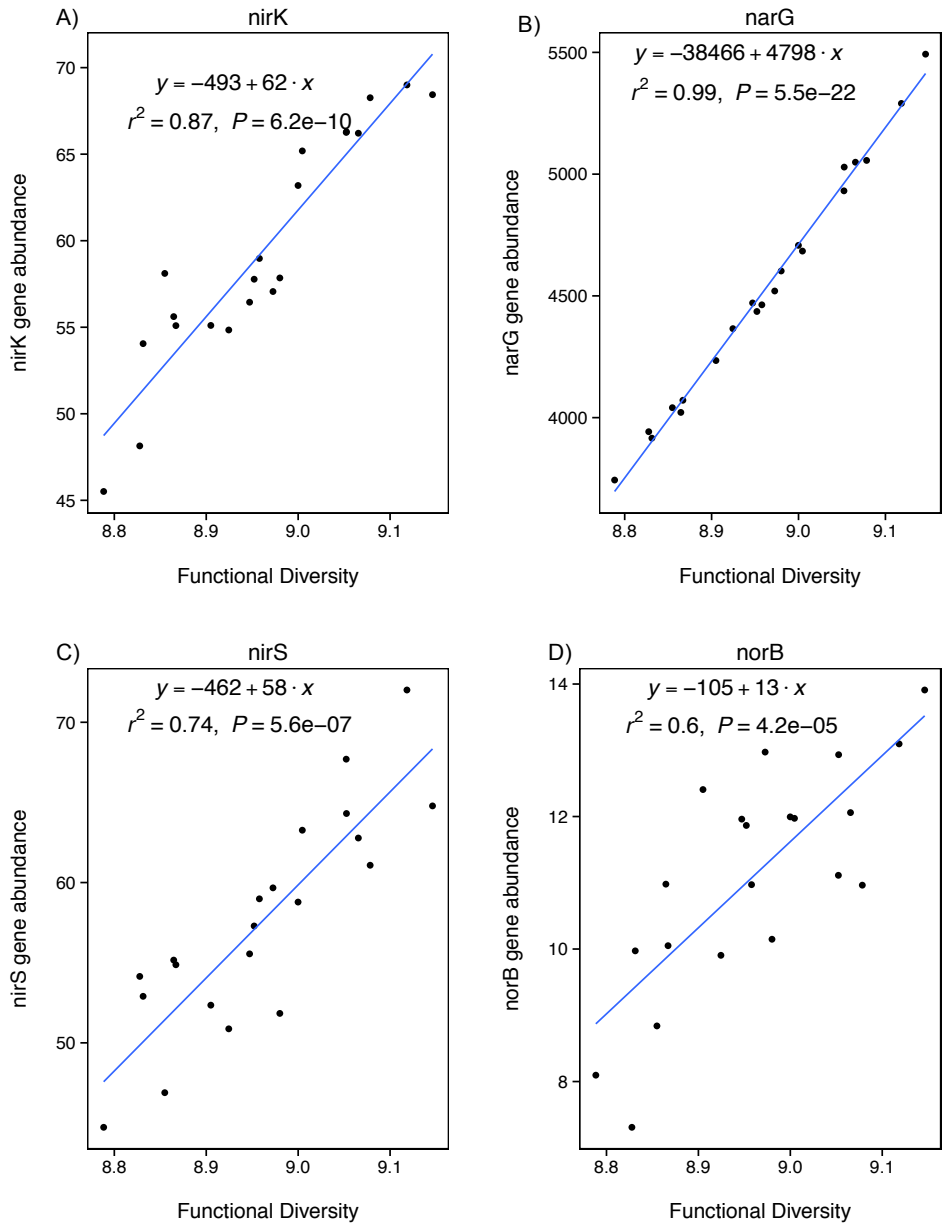


Figure 4. Functional diversity is positively correlated with changes in the abundances of specific genes involved in denitrification. These genes are involved in nitrite reduction (A: nirK, C: nirS), nitrate reduction (B: narG), and nitric oxide redunction (D: norB). We did not analyze nosZ because it is involved in a later stage of denitrification than included in our potential assay (nitrous oxide reduction).



CHAPTER 4: OPPOSING EFFECTS OF DIFFERENT SOIL ORGANIC MATTER FRACTIONS ON CROP YIELDS

In review at Ecological Applications

4.1 ABSTRACT

Soil organic matter is critical to sustainable agriculture because it provides nutrients to crops when decomposed and increases nutrient- and water-holding capacity when built-up. Fast- and slow-cycling fractions of soil organic matter can have different impacts on plant production because fast-cycling fractions rapidly release nutrients for short-term plant growth and slow-cycling fractions bind nutrients and build up water holding capacity. An emerging paradigm of soil organic matter dynamics suggests that these fast- and slow-cycling fractions are driven by different processes. We explored the controls on these fractions in tropical agroecosystems and their relationship to crop yields. We performed physical fractionation of soil organic matter from 48 farms and plots in western Kenya. We found that fast-cycling, particulate organic matter was positively related to crop yields while slower-cycling, mineral-associated organic matter was negatively related to yields. The positive effect of particulate organic matter was strongest in a low rainfall year, but insignificant when rainfall was abundant, suggesting that fast-cycling organic matter may be particularly important to maintaining yields in variable environments such as rain-fed tropical agriculture. Our finding that slower-cycling organic matter was negatively related to yield suggests the need for revisions to the paradigm that build up of mineral-associated organic matter positively impacts food security. Our results support a new paradigm of soil organic matter that different fractions respond to different underlying drivers and can have

different relationships with ecosystem services. Managing soils for sustainable agriculture will thus require quantifying the effects of specific organic matter fractions on ecosystem services.

4.2 INTRODUCTION

Soil organic matter (SOM) provides many benefits to people and ecosystems (e.g. Banwart et al. 2014, 2015). Organic matter is a particularly important component of soils in agricultural systems because it provides nutrients to crops when decomposed and increases nutrient holding capacity and water-holding capacity when built-up (Lal 2004). Despite these clear benefits, there are different viewpoints on how to manage SOM to support livelihoods. On the one hand are proponents of "using" SOM: encouraging decomposition of organic matter to liberate nutrients that can be taken up by crops in the short term (Janzen 2006). On the other hand are proponents of "saving" SOM by prioritizing the formation and long-term stabilization of organic matter to serve as a store for elevated atmospheric carbon (C) and enhance long-term soil quality through nutrient retention and water holding capacity (Lal 2004).

Although the ideas of "using" versus "saving" SOM seems, at the surface, to be at odds, an emerging paradigm of SOM dynamics suggests that these opposing processes occur in distinct components of SOM. Specifically, the fast-cycling particulate organic matter (POM) fraction serves as a source of nutrients to be "used" by plants, while the slow cycling, mineral-associated C fraction (MIN C) serves as a long term storehouse of C, "saved" in the soil for hundreds or thousands of years (Schmidt et al. 2011, Dungait et al. 2012). Under this emerging paradigm, nutrients for crop production are released from the decomposition of the fast-cycling, POM fraction, dominated by partially decomposed OM. Conversely, the slow-cycling, mineral-associated SOM fraction is primarily responsible for long-term storage of atmospheric CO_2, nutrient retention, and water holding capacity. This mineral-associated fraction is formed from C

compounds—often low molecular weight C from root inputs—which are assimilated by soil microbes, converted into microbial biomass, and stabilized on the charged surfaces of mineral soil particles in the form of microbial necromass and microbial exudates (Cotrufo et al. 2013). These microbial products may be the primary pathway through which plant inputs become bound to mineral surfaces and thus stabilized into long-term stores of SOM (Schmidt et al. 2011).

In addition to their role in the formation of new SOM, microbes also drive the decomposition and transformation of old SOM. However, most of the compounds in SOM cannot be directly assimilated by microbes; rather, they must first be oxidized and depolymerized by extracellular enzymes that are released into the soil environment by microbes (Burns et al. 2013). Microbial production of enzymes incurs both energetic and nitrogen (N) costs, and thus the allocation of resources is responsive to the elemental stoichiometry of available substrates (Sinsabaugh et al. 2008, Allison et al. 2011). As a result, enzyme activities can be interpreted as indicators of the relative availability of accessible C and nutrients, and should not be responsive to substrates that are minerally-protected and thereby not or minimally vulnerable to enzymatic attack.

Given the important roles of fast and slow cycling SOM fractions in sustainable agriculture, it is important to understand how agricultural management practices control the stocks of these two SOM fractions, and how in turn these stocks affect agroecosystem services such as crop productivity. This need is especially relevant to farming systems that have low external inputs, such as tropical smallholder agriculture, and thus depend on ecological processes, such as decomposition, to maintain soil fertility. Tropical smallholder agriculture supports the livelihoods of over 900 million of the world's poorest people (Wiggins et al. 2010). These peoples are clustered in many poor regions such as sub-Saharan Africa, where more than

90% of farms belong to smallholders (GRAIN 2014). These smallholder farms contribute the majority of food production in many poorer countries—in Kenya, for example, small farms produced 73% of agricultural output in 2004 (Binswanger-Mkhize et al. 2009).

The purpose of our research is to study how mineral and organic nutrient addition to smallholder farms in western Kenya impacts fast- and slow-cycling SOM fractions, the microbial extracellular enzymes responsible for OM decomposition, and resulting changes in crop yields. We studied smallholder farms in western Kenya that participate in the Millennium Villages Project (MVP) (Wood et al. 2015b, 2015a). The MVP aims to achieve the Millennium Development Goals in sub-Saharan Africa and includes an agricultural component that focuses on increasing crop yields through applications of mineral and organic fertilizers to redress negative soil nutrient balances—an approach often referred to as the African Green Revolution (Sanchez et al. 2007).

The degrading effects of continuous cultivation on soil quality can be attenuated by organic inputs (Moebius-Clune et al. 2011). We therefore expected that recent organic inputs would be an important control on SOM fraction sizes, the microbial mechanisms of decomposition of those fractions, and resulting yields. We hypothesized (H1) that the inclusion of organic inputs in a farm's management leads to lower activity of C-related enzymes because of abundant C and greater investment in N- and P-related enzymes. We predicted that recent organic inputs would be positively associated with the size of particulate organic matter fractions, but that the longer-term land use history, such as the vegetation history, would dictate the size of mineral-associated fractions, which respond over longer time scales (Lajtha et al. 2014). For relationships between the SOM fractions and yield, we hypothesized (H2) that POM stocks would be positively related to crop yields because POM represents a fast-cycling fraction

from which nutrients for crop growth can be liberated (Haynes 2005). We also expected higher crop yields with a larger stock of the slower-cycling, mineral-associated fraction because of its capacity to retain plant-available water and nutrients in the soil through binding of ions and polar molecules to positively- and negatively-charged sites on the OM (Lal 2004, Haynes 2005).

4.3 Materials and Methods

4.3.1 Study site and sample collection

Soils were collected from 24 experimental fertilization plots in Yala, Kenya and from 24 maize farms in the same area participating in the Millennium Villages Project (MVP). The fertilization plots were located at the field office of MVP (34.511 E, 0.101 N). They represent mineral fertilizer additions of 0, 50, 75, 100, 150, and 200 kg N ha^{-1} with four plots per fertilizer level and maize grown on the plots. Actively managed farms were distributed across the landscape surrounding the MVP and are clustered by treatment on similar underlying soils (Wood et al. 2015b, 2015a). The area was originally part of the moist broadleaf forest zone in eastern and central Africa, but is now a mixed-maize agricultural system, with most farmers cultivating a maize crop in both the long and short rainy seasons. Some farmers, however, replace the short-rain maize crop with a seasonal legume rotation that fixes N and is expected to build SOM. This treatment is henceforth referred to as legume rotation. Actively managed farms are then grouped into three categories: low fertilizer (<10 kg mineral N ha^{-1}); high fertilizer (>60 kg mineral N ha^{-1}); legume rotation (>60 kg mineral N ha^{-1} paired with legume rotation). Fertilizer is typically applied to the base of maize stalks with about 1/3 of N added at planting as diammonium phosphate and 2/3 applied 5-8 weeks later as urea. Greater detail of farms and selection criteria is given in Wood et al. (2015b, 2015a) and Tully et al. (in review). Fertilization plots and farms have broadly different land use histories. Fertilization plots are situated on land

owned by the Kenya Broadcasting Corporation and leased to MVP. The land was originally converted to agriculture in the 1960s or 1970s, but was left fallow in the 1980s and from 1994-2007. Fertilization plots were established in 2011. In contrast, the actively managed farms have been in production for at least 50 years. Greater detail of fertilization plots is given in Hickman et al. (in revision).

The mean annual temperature and precipitation for the study region are 24°C and 1800 mm, respectively. Annual precipitation is distributed bi-modally with 1120 mm in a long rainy season from March to June and 710 mm in a short rainy season from September to December. The soils are classified as Kandiudalfic Eutrodox (USDA classification) and are well-drained sandy clay loams (on average 53.75% sand, 12.59% silt, 33.54% clay) with a mean pH of 5.45 and C:N of 11.52 (0-20 cm; Table 1).

We sampled soils for this study in June 2013, in the middle of the long rains, two weeks after fertilizer application and when maize plants were nearing the pollination stage. For farms, we took 20 2-cm dia. soil cores from the top 20 cm of bulk soil. We targeted bulk soil to avoid changes in microbial community composition that could be due to direct impacts from crop root exudation as well as recent fertilizer application, which is targeted to the root zone. Cores were taken at regular intervals throughout an entire field area and homogenized and aggregated to a composite sample. Cores were spaced evenly across the field; the spacing of intervals thus depended on total field size. Because fertilization plots were significantly smaller than farms, we took nine 2-cm diameter cores to 20-cm depth per plot, aggregated to a composite sample. At each core location we recorded temperature and volumetric soil moisture content using a soil thermometer and a HydroSense moisture probe (Campbell Scientific, Logan, UT, USA).

4.3.2 General soil properties and crop yield

Subsamples of sieved (2-mm screen) field soil were stored at 4°C and used to determine pH, gravimetric soil moisture, and water holding capacity. Gravimetric soil moisture and water holding capacity (after wetting soils to field capacity) were determined by drying soil at 105°C for 24 h. Soil pH was determined using a benchtop meter and a 1:1 slurry of soil:H_2O by volume. We measured bulk density with a slide hammer (Core Sampler Complete, AMS Idaho, USA) by inserting a 5.08-cm diameter plastic tube into the corer, followed by a 10.12-cm depth aluminum tube (volume = 205.9 cm^3). The corer was driven into the soil to 20 cm; the aluminum tube was then removed, the soil leveled with a knife, and the whole tube was wrapped in aluminum foil. Aluminum tubes were weighed and oven-dried at 105°C until a constant weight was attained (Tully et al. in review).

Microbially available C was estimated using a 30-day C mineralization assay following Bradford et al. (2008) and Oldfield et al. (2014). Briefly, we measured CO_2-efflux four times across thirty days (days 1, 4, 15, 30). For each measurement, 4 g soil slurries were placed in 50 mL centrifuge tubes. Tubes were flushed with CO_2-free air and incubated for 24 h before determination of headspace CO_2 concentrations on an infrared gas analyzer (IRGA; Li-Cor Biosciences, Lincoln, NE, USA, Model LI-7000). Samples were maintained at 60% water holding capacity. Cumulative carbon mineralized was determined by integrating CO_2-efflux rate values across each measurement during the 30 days.

A subsample of sieved soil was air-dried and used to determine soil texture by the hydrometer method (Bouyoucos 1962). Yields were measured by harvesting aboveground biomass in a 3 m x 3 m sub-plot on actively managed fields and by harvesting the entire plot on

the experimental plot. Harvested plants were separated into stalks and cobs and weighed in the field. Subsamples were taken from the field, cobs separated into core and grain, and all materials weighed fresh and oven-dried (60°C until constant mass was obtained). Plot yields were estimated based on dry grain per plant and the total number of plants per plot; yields were then scaled to a per hectare basis. Further description is given in Tully et al. (in review).

4.3.3 Soil organic matter fractions

We used a size-based physical fractionation method to differentiate between the faster-cycling particulate organic matter (POM) and slower-cycling mineral-associated (MIN) soil C and N fractions (Schlesinger and Lichter 2001), using the method described in Bradford et al. (2008). Briefly, air-dried soil (10 g) from each plot was dispersed with sodium hexametaphosphate via shaking (18 h) to break apart aggregates, and then passed through a 53-μm sieve to physically fractionate the soil. Mineral-associated (i.e., silt and clay minerals) material is considered <53 μm and POM material is >53 μm. Both soil fractions were dried (65°C until constant mass achieved) and ball-milled to a fine powder. Carbon, N and ^{13}C contents were measured on a Costech ESC 4010 Elemental Analyzer (Costech Analytical Technologies Inc., Valencia, CA) coupled to a Thermo Delta Plus Advantage (San Jose, CA, USA) continuous-flow isotope ratio mass spectrometer in the Earth Systems Center for Stable Isotopic Studies at the Yale Institute for Biospheric Studies. Analytical precision was ±0.2 $\delta^{13}C$ ‰. Carbon isotope values are expressed relative to Pee Dee Belemnite (vPDB). We present estimates of the different soil C and N fractions per m^2 to a depth of 20 cm using bulk density (g cm^{-3}) measurements described above.

As maize is a C_4 species, it is enriched in $\delta^{13}C$ relative to the C_3-dominated native forest

vegetation. We were therefore able to use natural abundance stable isotopes to determine the relative proportion of total soil C from C_3 vs. C_4 sources, and thus estimate the proportion of soil C that is derived from recent maize agriculture vs. native vegetation and occasional cultivation of non-C_4 crops (Ineson et al. 1995). We determined the relative contribution of C_4- vs. C_3-derived C to total soil C fractions using stable isotope mixing equations (Fry 2007). We used the equation

$$C_{cultivated} = 100 * ({}^{13}C_{cultivated} - {}^{13}C_{native}) / ({}^{13}C_{maize} - {}^{13}C_{native})$$

where $C_{cultivated}$ is the C_4-derived fraction of the total soil C fraction, ${}^{13}C_{cultivated}$ is the $\delta^{13}C$ value of soil in plots where maize is present, ${}^{13}C_{native}$ is the $\delta^{13}C$ value of soil under native forest vegetation, and ${}^{13}C_{maize}$ is the $\delta^{13}C$ value of the maize stover itself. We used a literature-derived value from the nearby Kakamega Forest for the ${}^{13}C_{native}$ (-24.33, Awiti et al. 2008). To estimate the $\delta^{13}C$ of maize we collected and ball milled maize stover from two of the fertilization plots and used the average of these values as an estimate of maize $\delta^{13}C$ for our sites (Table S1).

4.3.4 Extracellular enzyme potential assays

Sub-samples of soil for enzymatic assays were frozen immediately after sampling and transported within one week to the United States where they were frozen at -20°C and transported on dry ice to the Natural Resource Ecology Laboratory at Colorado State University. While freezing can reduce measured enzyme activities, relative differences among treatments and sites persist (German et al. 2011). All enzyme assays were conducted within one month of sampling. We measured the rate of potential activity of seven hydrolytic enzymes (Table S2) using a fluorometric approach described in German et al. (2011) and Bell et al. (2013). Four of the measured enzymes (BG, CB, XYL, AG) are involved in degrading C-rich substrates, two

(NAG and LAP) are involved in degrading N-rich substrates, and one is involved in degrading P-rich substrates (PHOS).

Sub-samples of 2.75 g of fresh soil were homogenized and aliquots were pipetted into 96-well plate. Soils were inoculated with a non-limiting amount (200 µL) of each fluorescently labeled substrate (Table S2) dissolved in deionized water. The plate was inverted several times to mix soil samples and substrates and placed in an incubator at 25°C. Reference standards were prepared in a similar manner as the soil samples. In the standard plates, we added fluorescent standards, instead of the substrates, in seven concentrations: 0, 2.5, 5, 10, 25, 50, 100 µM. We used two types of fluorescent standards, 7-amino-4-methylcoumarin (MUC) and 4-methylumbelliferone (MUB); MUC standards were used for LAP, and MUB for the other enzymes.

After incubation, the plates were centrifuged and 250 µL of supernatant was removed from each well and pipetted into a corresponding well of a 96-well black plate. Fluorescent activities were immediately measured using an Infinite M500 spectrofluorometer (Tecan, Männedorf, Switzerland). Readings of the fluorescent activities from standards were used to calculate potential enzyme activities for each sample in nmol activity g^{-1} dry soil h^{-1}. We calculated three enzymatic stoichiometric ratios C:N, C:P, and N:P based on the main substrates related to each enzyme. The ratios are calculated by summing the log of enzyme potential activities for each enzyme within a given nutrient category (e.g., C) and dividing by the sum of the nutrients in the other category (e.g., N).

4.3.5 Data analysis

4.3.5.1 Soil organic matter fractions and extracellular enzyme potential activities

To determine the factors controlling the sizes of fast- and slow-cycling SOM fractions, we used a linear modeling approach with soil and treatment covariates as predictor variables and fraction size as a response variable. Initial predictor variables included pH, % clay, N addition, legume rotation, and a binary variable indicating whether an observation came from an experimental plot versus farm. We selected a final, reduced-form model that optimized adjusted R^2. To check for normality in the response variables we used a Shapiro-Wilk normality test. In cases where the normality assumption was violated we transformed response variables using a Box-Cox transformation before performing model selection (Mateu 1997). We tested for assumptions of constant variance and report robust standard error estimates in cases where this was violated. Validation of linear model assumptions was done using the *gvlma* package in the statistical freeware R (Pena and Slate 2010). We standardized model coefficients using a z-transformation in which we converted all model variables to a common mean and standard deviation by subtracting the mean and dividing by the standard deviation for all independent model variables (Gelman 2008). The approach gives model coefficients that describe the standardized slopes, which, unlike partial correlation coefficients, are comparable in magnitude within models because variables are expressed in common units (Schielzeth 2010). Standardization was done using the *arm* package in R (Gelman et al. 2009). For all statistical tests, we considered coefficients with $P < 0.05$ significant and coefficients with $P < 0.10$ marginally significant (Hurlbert and Lombardi 2009).

To assess the determinants of extracellular enzyme potential activity we used the same procedure described above. In addition to including soil properties and farm characteristics in the full, initial model we also included soil C fraction sizes as potential predictors of enzyme potential activity.

4.3.5.2 Organic matter relationship with crop yields

We used a non-linear least squares approach to model the relationship between yields, SOM fractions, and farm management and other soil properties. More specifically, we fitted exponential models defined by the equation:

$$y = a^{(k-x1)} + BX$$

where x_1 is the particular soil fraction (either POM or MIN C), *k* is the growth/decay parameter, B is a vector of coefficients, and X is a vector of control variables corresponding to B, including % clay, N addition, a binary variable for legume rotation, and a binary variable for whether an observation is from fertilization plots or actively managed farms. For a positive relationship between yield and SOM, an exponential growth model was fitted with a positive value for *k* and for a negative relationship an exponential decay model was fit with a negative value for *k*. Because conventional goodness-of-fit measures (e.g., R^2, AIC, etc.) are not applicable to non-linear models, we used a prediction-and-visualization procedure to assess overall model fit. We used our model to predict new data based on the parameters generated for the model. We visualized those predicted values on top of actual, observed data to indicate whether the functional form and spread of the data was close to the original data; high overlap between original and predicted data suggests an appropriate model.

4.3.5.3 Structural equation modeling

Because of conceptual linkages among different models (i.e., enzyme activity can be influenced by management and influence soil C fractions), we used structural equation modeling to simultaneously represent relationships among models and model variables. More specifically, we used structural equation models to simultaneously estimate each of the pathways among farm

management, microbial enzyme potential activity, soil C fractions, and yields while accounting for correlations between multiple response variables (Grace 2006). We also visualize our hypothesized relationships between these variables.

We report standardized path estimates that allow for comparison of the relative magnitude of variables within the same model (Grace and Bollen 2005). For model goodness-of-fit, we report X^2 and root mean square error of approximation (RMSEA). These measures assess the similarity between the covariance matrix of the observed data and the covariance matrix implied by the specified model. A X^2 P-value greater than 0.05 implies significant overlap between the observed and implied data, and thus adequate model fit. Because the X^2 test is based on large sample theory, we also report RMSEA, which is a goodness-of-fit measure weighted by sample size. We use an RMSEA value below 0.1 to represent good model fit because for sample sizes less than 50, the conventional RMSEA cut-off value of 0.05 is overly conservative (Chen et al. 2008). Individual paths were estimated using maximum likelihood. Insignificant paths were excluded from models unless they significantly improved overall model fit, based on X^2 and RMSEA values as well as assessment of modification indices (Grace 2006). Soil covariates (% clay and pH) were included in models where significant, but were not visualized in the results path diagram to minimize the number of arrows and improve readability; % clay and pH are included in reported model results. Where direction of effect could not be determined, paths are represented by dotted lines with double arrows and the thickness of the line represents the standardized correlation between the two variables rather than the standardized regression coefficient. All models were fitted using the *lavaan* package in R (Rosseel 2012).

4.4 RESULTS

4.4.1 Soil organic matter

4.4.1.1 Microbially available, particulate, and mineral-associated C fractions

Microbially available C fractions were 32% higher on actively managed farms (3.77 g C m^{-2} to 20 cm) than on fertilization plots (2.85 g C m^{-2} to 20 cm). Nitrogen addition was positively related to microbially available C fraction size (P=0.03, Figure 1C, Table S4). The standardized coefficient for the plot identity, however, was 2.3-times larger than the N addition coefficient and thus had 2.3-times greater effect on microbially available C fraction size than N addition.

Particulate organic matter (POM) C:N was, on average, 17.71 (Table 1). The plot binary variable was the only predictor of POM C:N (P=0.01, Table S5) with 7.85% higher C:N on fertilization plots (18.38) than actively managed farms (17.04). Particulate OM C was significantly related to legume rotation, pH, and N addition. POM C fractions were 22% greater on legume rotation fields (86.26 g C m^{-2} soil to 20 cm) than on non-legume rotation fields (70.42 g C m^{-2} soil to 20 cm, including both non-legume rotation fields and fertilization plots). Legume rotation also had the greatest effect on POM C, with an effect 1.11 times greater than pH and 1.13 times greater than N addition, both of which were also significantly, positively related to POM C fraction size (Figure 1A, Table S4).

Mineral-associated OM (MIN) C:N was, on average, 10.68 (Table 1). Soil pH was also positively, but not significantly, related to MIN C:N (Table S5). Mineral-associated C was significantly related to plot, pH, and % clay. Fraction sizes were 16.65% higher on fertilization plots (345.16 g C m^{-2} soil to 20 cm) than actively managed farms (295.90 g C m^{-2} soil to 20 cm). Plot had the greatest relative effect on MIN C, with an effect 2.83 times the size of the effect of pH, which was positively related to MIN C fraction size, and 4.04 times the size of the effect of % clay, though this effect was insignificant (Figure 1B, Table S4).

4.4.1.2 C_3-vs.-C_4-derived total soil organic matter

Plots and farms had, on average, 65% of total soil C derived from C_3 vegetation (35% of total C was C_4-derived, Table 1). The percent of C_3-derived total soil C was not significantly impacted by farm management, and was only significantly (and positively) related to soil pH, though overall model fit was low (Table S5). Soil C $\delta_{13}C$ values were also affected by pH, but not other variables, though again with a small effect and poor overall model fit (P=0.04, adj. R^2=0.07, Table S5). C_3-derived total C stocks, however, significantly varied by land use history. If an observation came from an experimental plot, then this significantly determined the total stock of C_3-derived C (Figure 1D, Table S4); plots had 14.57% higher stocks of C_3-derived total C than actively managed farms (Table 1). Soil pH was the strongest statistical predictor of C_3-derived C stocks (positively related) and had 1.25 times the effect of the plot binary variable (Figure 1D, Table S4).

4.4.1.3 Relationship with yields

Controlling for nutrient addition and underlying differences between plots and farms, we found that the POM C fraction was positively related to yield ($P < 0.1$), whereas MIN C fractions were negatively associated with yield ($p < 0.05$, Figure 2, Table 2). Percent clay was negatively related to yields (POM model: $P < 0.05$, MIN model: $P < 0.01$), while legume rotation and N addition were positively, but not significantly related to yields (Table 2).

4.4.2 Extracellular enzyme potential

The potential activity of all C-related enzymes was significantly impacted by several factors, including pH and % clay, legume rotation and the experimental plot binary variable, and the size of microbially available and particulate C fractions (Figure 3A, All individual enzyme

activities reported in Table S3). The effect of plot was greatest with C-enzyme potential activity being 24.61% lower on fertilization plots than actively managed farms ($P<0.01$). Microbially available C was also negatively related to C-enzyme activity ($P=0.03$), while POM C was positively related to activity ($P=0.07$) and % clay was negatively related ($P=0.05$, Figure 3A).

Potential activities of C- vs. N-degrading enzymes were 25.49% lower on fertilization plots compared to actively managed farms ($P=0.01$). Microbially available C was negatively related to the relative activity of C- vs. N enzymes ($P=0.09$) and this effect was 4.26 times less than the effect of the plot binary variable (Figure 3B). Both % clay and MIN C were negatively related to the investment in C- vs. P-degrading enzymes ($P<0.01$ and $P=0.02$, respectively), with the effect of % clay 14.43 times greater than the effect of MIN C (Figure 3C). The model of N- vs. P degrading enzymes, by contrast, had low explanatory power (adj. $R^2=0.09$) and only % clay was significantly related to enzymatic stoichiometry (Figure 3D).

4.4.3. Structural equation models

Our structural equation modeling confirmed results from the separate linear and non-linear models (Figure 4B, Table 3). We found that yields in 2013 depended positively and significantly on POM, but negatively on MIN C. The SEM allowed us to assess the relative importance of these variables, with MIN C having the strongest effect, the plot binary variable the second strongest, POM the third, and % clay the smallest effect. Particulate OM C fractions were significantly elevated under N addition and legume rotation with N addition having the largest effect. Though yields depended on POM and POM depended on N addition and legume rotation, N addition and legume rotation did not affect yields other than through POM in 2013 (though there was a relationship in 2012: see below for results and explanation in discussion).

Mineral-associated C fractions were significantly higher, and microbial available C lower, on fertilization plots. Mineral-associated C was significantly correlated with total C enzymes, but the direction of the effect (whether enzymes impact C fractions or vice versa) could not be determined. Microbially available C depended significantly and positively on N addition.

To test the robustness of our results across time, we re-fit SEMs using nutrient addition and crop yield data from 2012. We found that in 2012 nutrient addition and legume rotation significantly, positively affected yields, consistent with what would be expected with other analyses (Figure S1, Wood et al. 2015b). The positive effect of POM on yields observed in 2013 diminished and was only marginally significant. We still observed a strong, significant negative relationship between MIN C and crop yield. All other patterns were broadly similar to 2013.

4.5 DISCUSSION

The objective of our study was to identify the effect of organic and mineral nutrient addition on microbial enzyme activities—the proximate mechanism of SOM transformation and degradation—and on SOM fractions and their relationship to yield. In support of our first hypothesis, we found that the inclusion of organic inputs leads to lower activity of C-related enzymes. These recent organic inputs were also positively associated with the size of POM fractions, potentially due to lower investment of microbes in degradation because of abundant C. Longer-term presence of vegetation was instead the dominant control of mineral-associated fractions, which turn over on longer time scales.

In support of our second hypothesis, we found that fields with higher POM had greater yields independent of nutrient addition. However, this effect was only significant in a year with variable rainfall; when rainfall was constant throughout the growing season mineral nutrient

addition, not POM, was the dominant driver of plant yield. Even more surprisingly, we found that mineral-associated C fractions were negatively correlated with yields. This finding is in conflict with the notion that the long-term build up of SOM, which is dominated by mineral-associated fractions in most agricultural soils, can contribute to food security (Lal 2004 but see Janzen 2006). It suggests that further research is needed to identify and quantify the effect of particular OM fractions on plant production.

4.5.1 Changes in enzyme stoichiometry

Because microbial enzymatic activity is the proximate mechanism of SOM transformation, differences in microbial functional capacity, such as enzymatic capacity and activity, may help predict changes in SOM pools due to management or environmental change (Allison et al. 2010, Wood et al. 2015b). Previous findings have shown that the stoichiometric ratio of extracellular enzyme acquisition is responsive to nutrient availability (Sinsabaugh et al. 2008). Consistent with this, and with our first hypothesis, we found that enzymes involved in the degradation of C-rich substrates were less active in soils that had greater soil C pools and were associated with treatment categories that were associated with greater soil C (e.g. legume rotation, fertilization plots). Our finding that enzymatic potential of C-related enzymes is lower in soils with organic inputs suggests that investment in C-degrading enzymes in C-rich environments may be an important indicator of broad-scale differences in C stocks. It may then be a useful predictor of longer-term changes in soil C pools before these effects emerge over longer time scales (Conant et al. 2011) and, as a result, potential changes in crop yield due to differences in these C pools. Other work has found that N addition to N-limited systems can stimulate OM decomposition through changes in enzymatic ratios (Keeler et al. 2008). We did not find that enzyme ratios—either C:(N or P) or N:P—were dependent on N addition.

4.5.2 Organic matter fractions and crop yields

Confirming our first hypothesis, we found that short-term organic inputs (legume rotation) were positively related with the POM fraction and that the longer-term fallow on fertilization plots was strongly related with the mineral-associated fraction. To test our second hypothesis regarding the effects of these different pools on yields, we used structural equation modeling to control for nutrient addition and soil properties and found that POM C was positively related to yields. This finding confirmed our hypothesis that POM would be positively related to crop yields because it represents a faster-cycling fraction that can liberate nutrients for crop growth (Haynes 2005).

Despite the direct effects of POM on yields, we found that short-term nutrient addition through legume rotation and N addition did not directly impact crop yield, which was counter to our expectation. Though counterintuitive, this finding is consistent with other results from this system which show that crop yields were not impacted by N addition in 2013, but were impacted by nutrient input in 2012 (Tully et al. in review). One possible explanation for this finding is poor rain conditions in 2013. In 2013, weather was variable and droughts were frequent at important crop growth stages (Tully et al. in review), which may have acted as an overall control on crop productivity, masking the effect of nutrient addition. In 2012 there was a more favorable climate and SEM results show that yields were significantly related to both legume rotation and N addition. The positive effect of POM C was smaller and only marginally significant in 2012, when short-term nutrient input was significantly related to yields. This suggests that POM may not always be the strongest overall predictor of crop yield, but may play an important role in maintaining yields in variable environments such as rain-fed smallholder agriculture in the tropics.

In contrast to expectations under our second hypothesis, we found that mineral-associated fractions were significantly negatively related to crop yield in both variable- and constant-rainfall conditions. This finding conflicts with the current paradigm that long-term build up of SOM is important for food security (Lal 2004). Instead, our findings suggest that the effect of OM on productivity depends on the specific fraction of OM considered: build-up of short-term OM pools can be key to food security, especially in variable environments, but build-up of long-term pools may have a detrimental effect. Because these findings are based on observational data, experimental research is needed to identify the underlying mechanisms. For instance, increased POM may drive greater crop yields in variable rainfall conditions because of greater soil water aggregate formation and retention (Haynes 2005). The mechanism explaining the negative yield-mineral C relationship, however, is not obvious and demands immediate recent attention to ensure that managements to improve soil fertility that focus on building up long-term stores of SOM are in fact effective.

4.5.3 Fraction of native-derived soil carbon

After more than 50 years of continuous cultivation, 65% of total soil C was derived from non-maize, C_3 vegetation. Based on results from a nearby protected forest (Kakamega Forest), the switch-over in dominance between native and maize-derived C should occur at around 40 years after cultivation and the mean residence time of C_3-derived C is around 60 years (Awiti et al. 2008). The slower switch-over observed in our study compared to Awiti et al (2008) suggests that there may be context dependencies in the rate of turnover of bulk soil C in different tropical agroecosystems. Further work should determine the variability in and controls on residence time of soil C under cultivation in tropical agroecosystems. This understanding will be essential to developing agricultural management strategies that target the build up or break down of

particular soil C fractions for ecosystem services, such as C storage, crop production, water holding capacity, and other ecosystem services.

4.6 Conclusion

A key finding of our work is that yields were positively related to fast-cycling OM under unfavorable climate conditions, but not significantly related to yields in a year with favorable rainfall. Thus POM fractions may play an important role in maintaining yields through time in variable environments, such as rain-fed smallholder agriculture in the tropics. Future work should identify the mechanism behind such effects, such as potential changes in soil moisture under higher OM. Quantifying these mechanisms and their relationships to key outcomes, such as yield, will help develop agricultural strategies for smallholder farmers that may be more resilient to climate change.

The current focus on the benefits of SOM is broad and rarely distinguishes between different fractions (e.g. Banwart et al. 2014, 2015). Our results support the notion that different soil C pools have differential impacts on ecosystem services—in this case, positive impacts of POM and negative impacts of mineral-stabilized C on maize yield. This is a key finding because an emerging paradigm in SOM research suggests that different soil C pools may have different underlying drivers and potentially effects (Janzen 2006, Schmidt et al. 2011). Thus, understanding the controls on each of these pools and quantifying their impacts on different ecosystem properties will be essential to the management of SOM for ecosystem services.

Current paradigms in SOM management suggest that the long-term build up of SOM will have benefits for food security and climate change mitigation (Lal 2004). Our results challenge this paradigm by showing a negative relationship between yield and long-term stabilized OM.

Future work should clarify the mechanisms behind our finding. Nevertheless, it highlights that different SOM fractions may have differential relationships to key ecosystem services and that this context dependency needs to be taken into account when managing for soil-based ecosystem services.

4.7 TABLES AND FIGURES

4.7.1 Tables

Table 1. Soil properties, organic matter fractions, and enzyme stoichiometry by treatment. Individual enzyme activity is presented in Table 6. Values are means with standard deviation in parentheses.

	Soil Organic Matter										**Enzyme Stoichiometry**		
	C pools (g C m^2 $soil^{-1}$ to 20 cm depth)			*δ13C Values*			*C:N*			*%*	*nmols g dry $soil^{-1}$ h^{-1}*		
	Microbially-available	Mineral	Particulate	Mineral	Particulate	Total	Mineral	Particulate	Total	Non-maize C	C:N	C:P	N:P
Managed Farms													
Low Fertilizer (n=9)	2.93	308.03	65.94	-19.55	-21.84	-19.97	10.65	17.02	11.39	64.65	1.64	0.46	0.27
<10 kg N ha-1	[0.54]	[47.68]	[20.09]	[0.78]	[1.55]	[0.73]	[0.54]	[1.62]	[0.41]	[5.89]	[0.99]	[0.33]	[0.04]
High Fertilizer (n=9)	4.70	292.37	66.81	-18.84	-20.69	-19.18	10.56	16.86	11.33	58.22	1.08	0.32	0.31
> 60 kg N ha-1	[1.15]	[33.61]	[15.26]	[0.81]	[0.51]	[0.76]	[0.35]	[1.67]	[0.43]	[6.14]	[0.28]	[0.03]	[0.06]
High + Legume Rotation (n=6)	4.04	284.83	86.26	-19.02	-21.54	-19.59	10.49	17.61	11.57	61.58	1.22	0.33	0.30
	[0.89]	[42.07]	[24.62]	[2.08]	[2.14]	[2.00]	[0.34]	[1.66]	[0.59]	[16.19]	[0.45]	[0.07]	[0.09]
Experimental Plots													
(kg N ha^{-1})													
0 (n=4)	2.47	338.18	66.59	-19.69	-21.65	-20.02	10.72	17.50	11.44	65.35	1.22	0.34	0.29
	[0.45]	[50.86]	[18.88]	[1.06]	[0.96]	[0.95]	[0.59]	[1.55]	[0.45]	[8.05]	[0.47]	[0.11]	[0.08]
50 (n=4)	2.59	330.94	77.32	-19.51	-20.98	-19.75	10.63	18.66	11.56	62.66	1.01	0.31	0.33
	[0.32]	[24.27]	[37.47]	[0.44]	[0.73]	[0.51]	[0.66]	[1.05]	[0.91]	[2.92]	[0.14]	[0.11]	[0.17]
75 (n=4)	2.65	350.22	72.26	-19.35	-21.17	-19.64	10.82	18.99	11.69	61.76	0.96	0.26	0.28
	[0.52]	[34.56]	[5.30]	[0.56]	[0.57]	[0.57]	[0.23]	[1.38]	[0.19]	[4.96]	[0.18]	[0.05]	[0.04]
100 (n=4)	2.65	353.63	68.53	-19.23	-21.03	-19.52	10.70	18.09	11.46	61.01	0.86	0.26	0.32
	[0.37]	[26.93]	[3.60]	[0.20]	[0.33]	[0.18]	[0.36]	[0.10]	[0.29]	[1.44]	[0.10]	[0.08]	[0.14]
150 (n=4)	4.31	357.09	75.60	-19.76	-20.98	-19.97	10.93	19.36	11.83	62.84	1.17	0.27	0.25
	[2.98]	[21.73]	[11.12]	[0.78]	[0.72]	[0.76]	[0.62]	[0.60]	[0.53]	[5.09]	[0.49]	[0.06]	[0.05]
200 (n=4)	2.55	325.45	84.41	-18.64	-20.93	-19.14	10.42	17.63	11.38	62.78	1.01	0.29	0.30
	[0.42]	[17.76]	[12.70]	[1.25]	[0.74]	[1.19]	[0.34]	[0.75]	[0.29]	[11.94]	[0.21]	[0.05]	[0.09]

Table 2. Nonlinear model of crop yield response to different organic matter fractions. In the model formula x_1 is the variable of interest (the soil organic matter fraction) and *k* its parameter. X is a vector of control variables and B its associated parameter vector. * p < 0.1; ** p < 0.05; *** p < 0.001; **** p < 0.0001

	Yield	
	(1)	(2)
Model	$y = a^{(k*x_1)}+B*X$	
Parameters		
a	5.110*** (1.476)	11.326**** (2.983)
POM C (k)	0.002* (0.001)	
MIN C (k)		-0.002** (0.001)
Legume rotation	0.516 (0.558)	0.553 (0.517)
Plot vs. Farm	-1.772**** (0.381)	-1.080** (0.445)
% clay	-0.081** (0.040)	-0.110*** (0.039)
N Addition	0.001 (0.003)	0.001 (0.003)

Table 3. Parameter estimates and model statistics for structural equation model of the relationship between farm and soil properties, microbial enzymes, soil organic matter fractions, and crop yields. X^2 statistics represent overlap between observed- and model-implied data; P > 0.05 thus indicates that the model adequately represents the data. Root mean square error of approximation (RMSEA) is a sample-size weighted measure of model fit. A 90% confidence interval is reported; P values below 0.1 indicate good model fit.

	Estimate	SE	Standardized Estimate	P
Yield ~				
MIN C	-0.02	0.00	-0.41	0.00
Plot	-1.13	0.24	-0.35	0.00
POM C	0.02	0.01	0.25	0.00
Texture	-0.10	0.03	-0.28	0.00
POM C ~				
Agroforestry	16.58	6.94	0.28	0.02
N Addition	0.12	0.04	0.40	0.00
pH	19.22	8.08	0.44	0.02
MIN C ~				
pH	24.70	12.37	0.25	0.05
Plot	47.25	9.85	0.57	0.00
Microbial C ~				
N Addition	0.01	0.00	0.30	0.02
Plot	-1.12	0.30	-0.44	0.00
Total C Enzymes ~				
Plot	-39.52	16.56	-0.27	0.02
Texture	-5.00	2.57	-0.29	0.05
Total C Enzymes ~~				
Microbial C	-16.79	8.74	-0.22	0.06

Structural Equation Model Metrics	
n	48
df	35
χ^2	15.16
$P_{\chi 2}$	0.71
RMSEA	[0.00,0.10]
P_{RMSEA}	0.81

Table 4. Mean carbon, nitrogen, and carbon isotope values for different components of the maize plant. We assumed that husks, stems, and roots are the main components entering into the soil organic matter fraction and that they enter in equal parts.

	%C	%N	$\delta_{13}C$
Husks	41.6	0.2	-12.3
Silks and Tassels	41.8	1.6	-11.3
Stems	42.6	0.2	-12
Leaves	37.8	0.8	-12.3
Roots	41.1	0.4	-11.6

Table 5. Enzymes studied, the most relevant nutrient, and their particular function.

Enzyme	Abbreviation	Related Nutrient	Function
β-Glucosidase	BG	Carbon	Releases glucose from cellulose
Cellobiohydrolase	CB	Carbon	Releases disaccharides from cellulose
Xylosidase	XYL	Carbon	Degrades hemi-cellulose
α-Glucosidase	AG	Carbon	Releases glucose from soluble saccharides
N-acetyl-glucosaminidase	NAG	Nitrogen	Degrades chitin
Leucine-amino-peptidase	LAP	Nitrogen	Degrades protein into amino acids
Phosphatase	PHOS	Phosphorus	Releases phosphate ions from phosphate group

Table 6. Activities of individual enzymes included in assay.

	Individual Enzymes *nmols g dry soil^{-1} h^{-1}*						
	β Glucosidase	Cellobiohydrolase	N acetyl glucosaminidase	Phosphatase	Xylosidase	α Glucosidase	Leucine amino peptidase
Managed Farms							
Low Fertilizer	122.61	90.43	41.96	598.98	21.15	15.37	117.00
	[63.63]	[93.08]	[26.23]	[240.47]	[12.88]	[14.91]	[37.50]
High Fertilizer	108.10	45.02	49.03	583.95	18.21	13.24	127.50
	[26.52]	[10.11]	[26.60]	[98.56]	[6.73]	[5.24]	[18.54]
High + Legume rotation	109.69	41.85	31.09	549.09	15.44	14.22	128.01
	[32.26]	[13.65]	[4.89]	[97.10]	[4.65]	[5.61]	[41.01]
Fertilization plots							
0	99.71	46.93	31.32	557.11	15.04	11.04	118.90
	[50.00]	[27.72]	[16.65]	[251.54]	[9.39]	[5.57]	[40.46]
50	106.17	36.00	51.94	625.61	16.71	18.82	123.96
	[17.59]	[3.39]	[37.45]	[230.78]	[2.75]	[12.99]	[40.38]
75	94.93	37.86	27.87	621.07	14.38	18.04	141.45
	[28.85]	[7.93]	[10.05]	[127.37]	[3.13]	[14.88]	[11.76]
100	98.12	38.13	37.35	683.95	20.20	13.71	163.73
	[13.10]	[7.37]	[21.83]	[173.70]	[9.44]	[6.19]	[37.89]
150	84.83	35.01	28.43	553.04	17.18	15.93	108.98
	[26.80]	[9.72]	[10.49]	[108.11]	[9.29]	[7.05]	[35.57]
200	82.65	35.46	34.25	548.55	17.53	15.55	120.21
	[8.48]	[6.15]	[19.95]	[150.68]	[4.66]	[12.18]	[25.12]

Table 7. Regression results for organic matter and microbial enzyme models. Standardized regression coefficient estimates are reported with standard errors below in parentheses. *P<0.1; **P<0.05; ***P<0.01; ****P<0.001

	Organic Matter				**Enzymes**			
	POM C	*MIN C*	*Microbial C*	*C_3-derived C stock*	*Total C enzymes*	*C:N*	*C:P*	*N:P*
Plot		51.40****	-0.39****	31.31**	-0.02***	-0.23***		
		(9.99)	(0.07)	(13.04)	(0.01)	(0.08)		
Legume rotation	1.96**				-0.02			
	(0.85)				(0.01)			
N Addition	1.73***		0.17**					
	(0.63)		(0.07)					
Microbial C					-0.01**	-0.14*		0.21
					(0.01)	(0.08)		(0.21)
MIN C							-0.53**	
							(0.21)	
POM C					0.01*			
					(0.01)			
% clay		-12.71			-0.01*		-0.79****	-0.37*
		(10.18)			(0.01)		(0.21)	(0.21)
pH	1.77***	18.18*		39.22***	-0.01	-0.08		0.30
	(0.64)	(9.75)		(13.18)	(0.01)	(0.08)		(0.21)
Adj. R^2	0.20	0.39	0.37	0.23	0.24	0.14	0.28	0.09

Table 8. Regression results for other soil organic matter properties. Standardized regression coefficient estimates are reported with standard errors below in parentheses. *P<0.1; **P<0.05; ***P<0.01; ****P<0.001

	POM C:N	**MIN C:N**	**% C_3-derived C**	**$d^{13}C$ values**
Plot	1.29***	0.20		
	(0.47)	(0.13)		
Legume rotation	0.72			
	(0.64)			
pH	0.70	0.20	0.03**	0.00**
	(0.46)	(0.13)	(0.01)	(0.00)
N Addition	0.53			
	(0.49)			
Intercept	17.71****	10.68****	-0.28****	0.50****
	(0.19)	(0.07)	(0.01)	(0.00)
Adj. R^2	0.20	0.07	0.09	0.07

4.7.2 Figures

Figure 1. Coefficient plots for SOM. Standardized regression coefficients are visualized. Variables are stacked vertically according to relative impact on the response variable, with the most important variable at the top. Full model results are presented in Table 7.

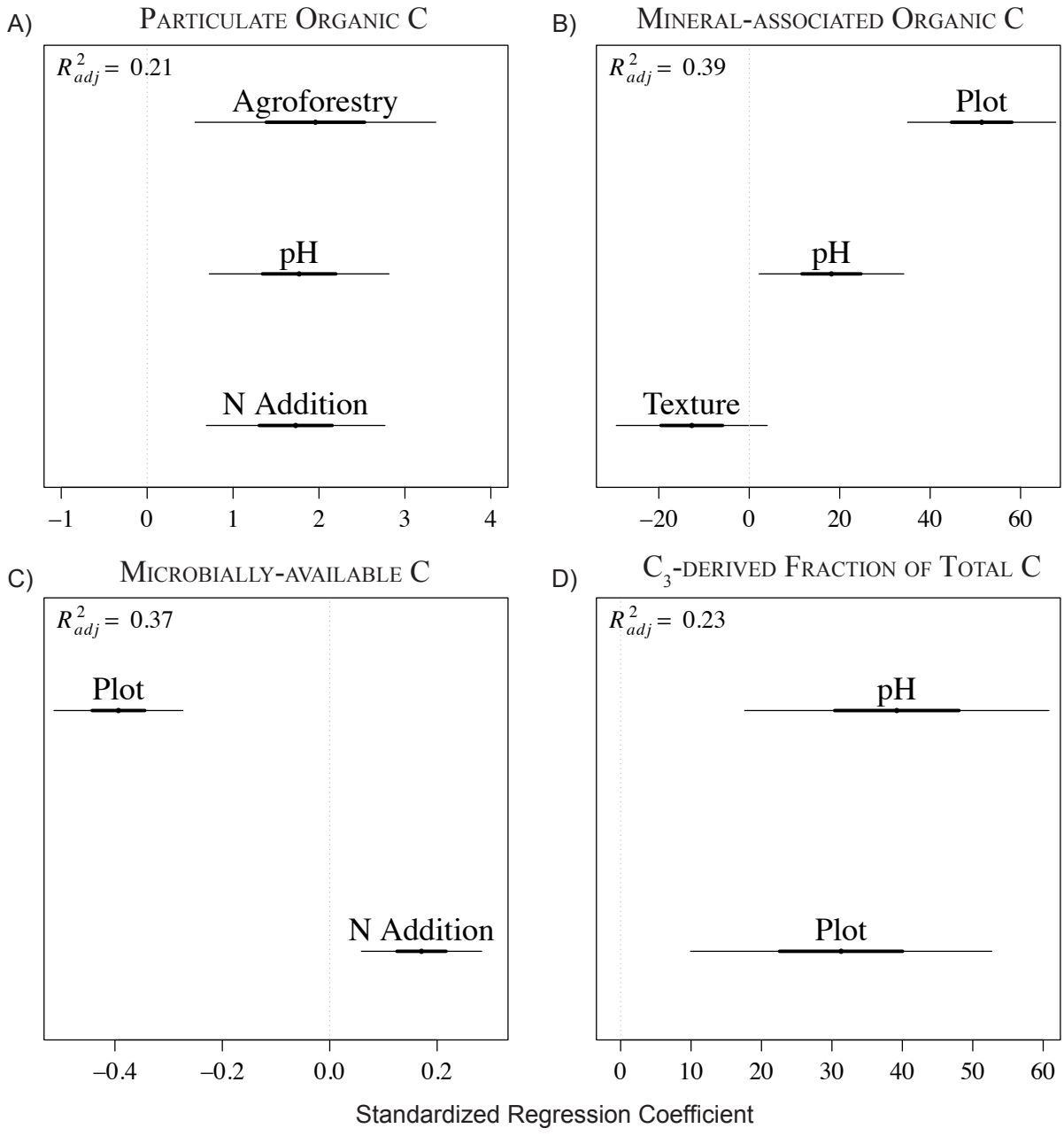


Figure 2. Yield increases with particulate organic matter (A) and decreases with mineral-associated organic matter (B). Filled circles are actual data points (n=48) and size is adjusted by percent clay content to visualize an important control variable. Unfilled circles are points predicted by the full nonlinear model. Equation is the equation of the line. Nonlinear model results are reported in Table 2.

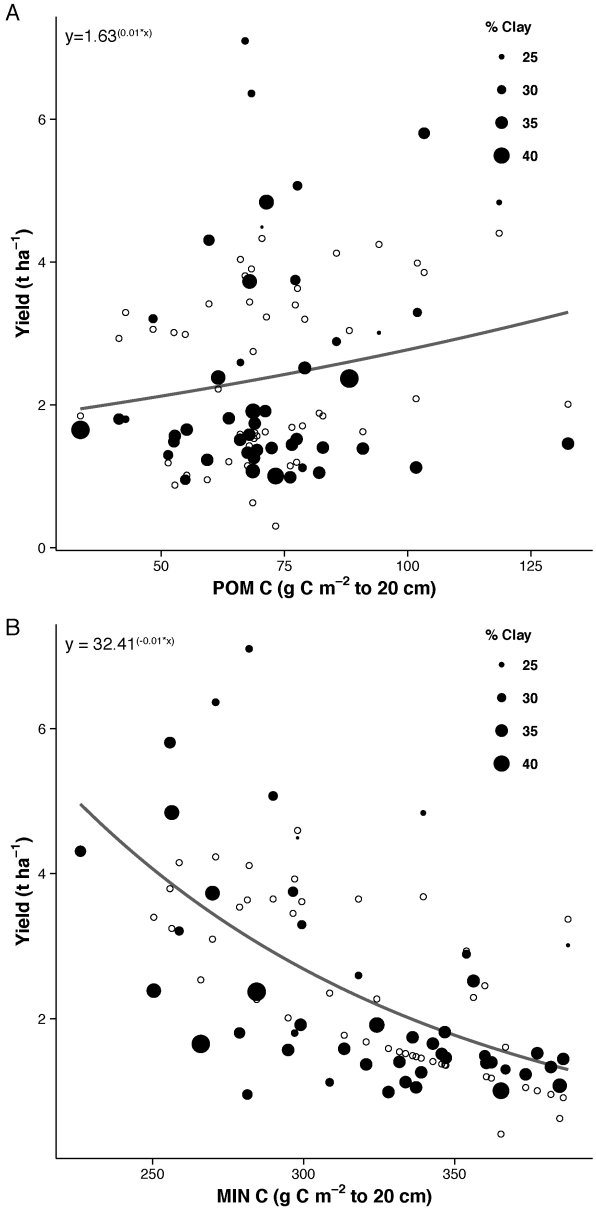


Figure 3. Coefficient plot for enzymes. Standardized regression coefficients are visualized. Variables are stacked vertically according to relative impact on the response variable, with the most important variable at the top. Full model results are presented in Table 7.

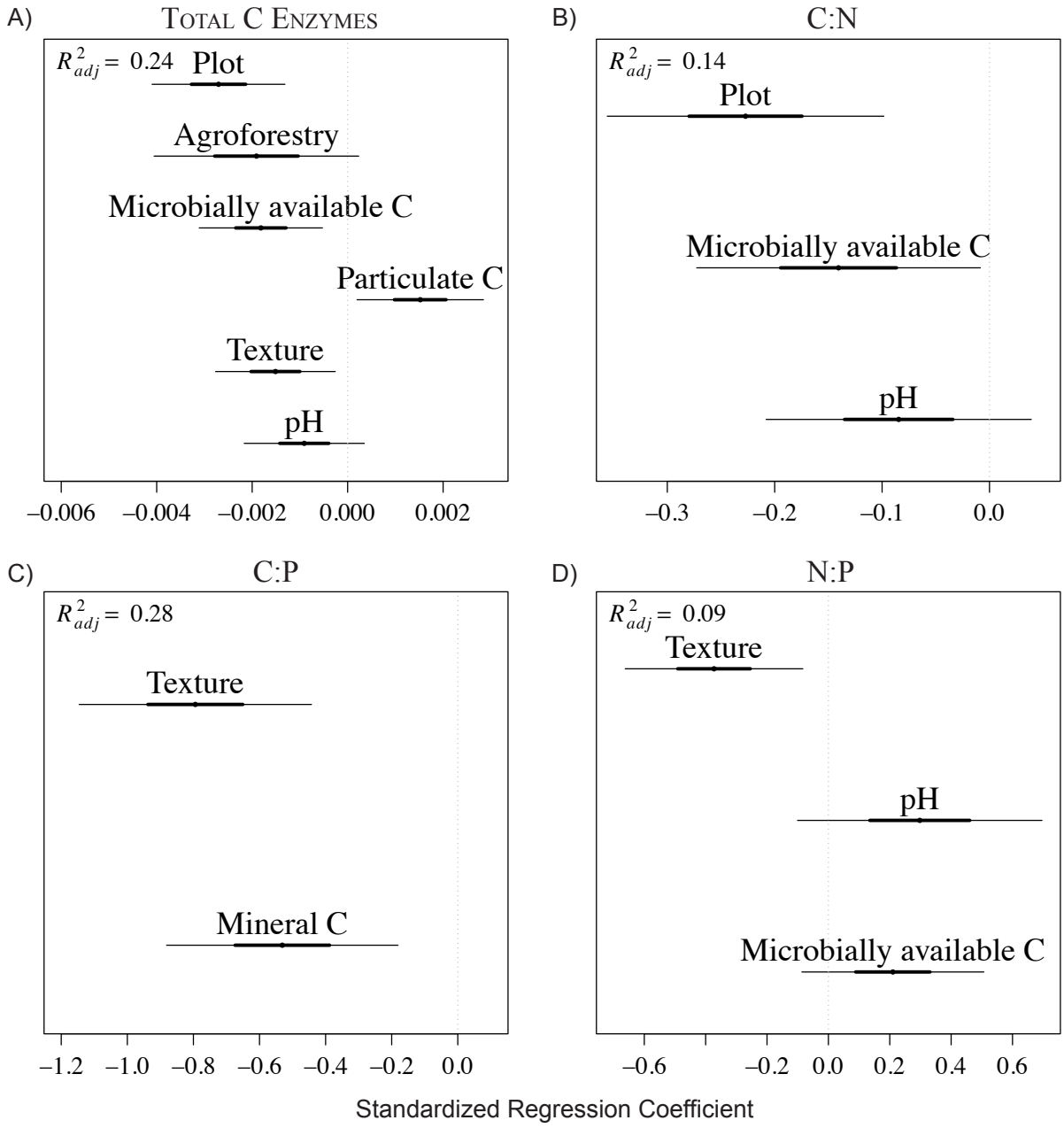


Figure 4. Structural equation modeling of the relationship between farm properties, microbial enzymes, soil organic matter fractions, and crop yields. Dotted lines indicate hypothesized relationships between variables (A). Solid lines (B) represent fitted relationships in the model that are statistically significant at $P < 0.05$. Shade represents effect direction (black = positive, gray = negative). Path widths are proportional to standardized regression coefficients, which are shown in Table 2 with P values and model statistics. Where direction of effect could not be determined, paths are represented by dotted lines with double arrows and the thickness of the line represents the standardized correlation between the two variables rather than the standardized regression coefficient. To visualize farm influence on enzymes, organic matter, and yield, models control for, but do not visualize, effects of pH and % clay (Table 2).

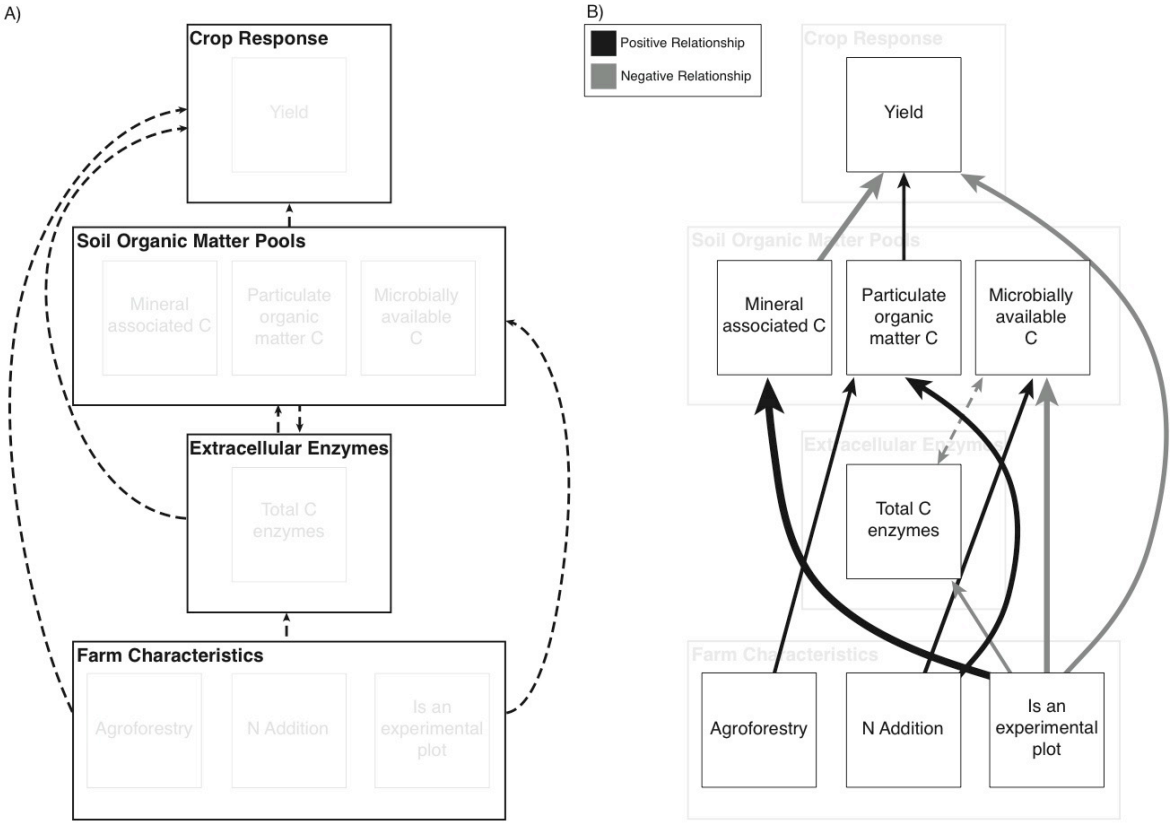
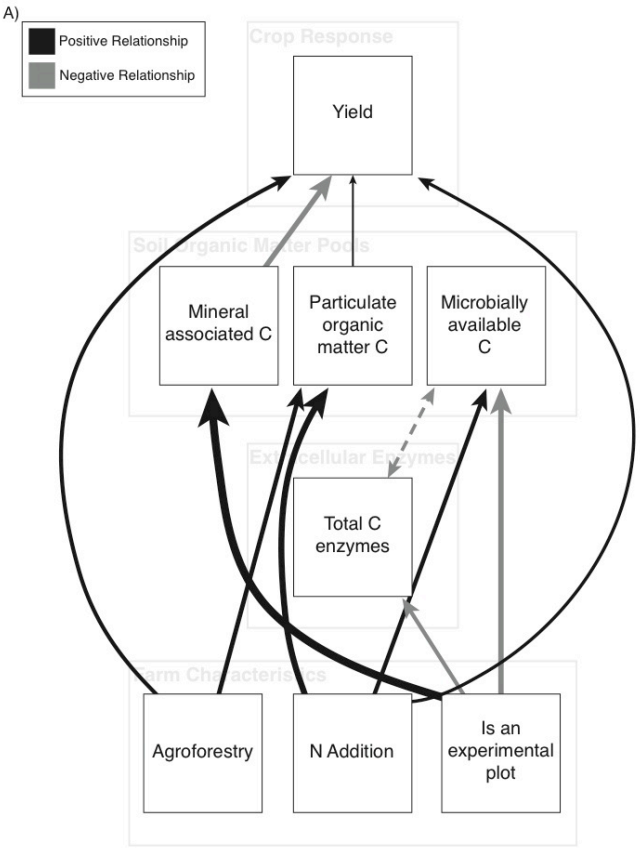


Figure 5. Structural equation modeling of the relationship between farm properties, microbial enzymes, soil organic matter fractions, and crop yields using yield and N addition data from 2012. Solid lines (A) represent fitted relationships in the model that are statistically significant at $P < 0.10$. Shade represents effect direction (black = positive, gray = negative). Path widths are proportional to standardized regression coefficients, which are shown in panel B with p-values and model statistics. Where direction of effect could not be determined, paths are represented by dotted lines with double arrows and the thickness of the line represents the standardized correlation between the two variables rather than the standardize regression coefficient. To visualize farm influence on enzymes, organic matter, and yield, models control for, but do not visualize, effects of pH and % clay (B).

A)



B)

	Estimate	SE	Standardized Estimate	P
Yield ~				
Agroforestry	1.21	0.47	0.29	0.01
N Addition	0.01	0.00	0.24	0.01
MIN C	-0.01	0.00	-0.37	0.00
POM C	0.01	0.01	0.14	0.09
POM C ~				
Agroforestry	19.43	7.43	0.33	0.01
N Addition	0.14	0.05	0.44	0.00
pH	20.60	8.56	0.47	0.02
MIN C ~				
pH	24.70	12.37	0.25	0.05
Plot	47.25	9.85	0.57	0.00
Microbial C ~				
N Addition	0.01	0.00	0.31	0.03
Plot	-0.99	0.23	-0.39	0.00
Total C Enzymes ~				
Plot	-42.34	16.29	-0.29	0.01
Texture	-5.10	2.56	-0.30	0.05
Total C Enzymes ~~				
Microbial C	-18.95	8.76	-0.25	0.03

Structural Equation Model Metrics

n	48
df	35
χ^2	13.11
$P_{\chi 2}$	0.83
RMSEA	[0.00,0.07]
P_{RMSEA}	0.90

CONCLUSION

The objective of this thesis was to determine if efforts aimed at increasing crop yields on smallholder African farms have consequences for the diversity of soil microbial communities and their impact on ecosystem processes that feedback to agriculture, such as soil carbon (C) and nitrogen cycling. Below I synthesize three major themes of results and reflect on future research priorities.

THE IMPORTANCE OF VEGETATION

In each empirical chapter, vegetation was significantly related to the response variables of interest. Legume rotations were significantly related to elevated microbial functional capacity (chs. 2 & 3) and were the strongest driver (positive) of potential denitrification and C mineralization—independent of legume-induced changes in microbial communities (ch. 3). Legume rotations were also significantly positively related with particulate soil organic matter (SOM) pools and significantly negatively related with C-related extracellular enzymes (ch. 4). Long-term differences in the presence of vegetation—represented by different fallow histories on experimental plots than actively managed farms—significantly suppressed microbially available C and C-related enzymes and was significantly positively related with stable SOM pools (ch. 4).

These findings regarding the importance of vegetation to soil microbial communities and processes correspond with other research showing that soil microbial communities are sensitie to changes in vegetation, such as forest loss and conversion to agriculture (Bossio et al. 2005, Rodrigues et al. 2013, Crowther et al. 2014). Such changes in belowground communities are driven by vegetative traits (de Vries et al. 2012, Zancarini et al. 2013). Yet no studies have quantified the linkages between vegetative traits and the functional composition of belowground communities in arable system. Future research should focus on identifying the mechanisms of

interactions between aboveground-belowground linkages in arable systems. In chapter 1, I advocated for a trait-based research agenda for arable systems, which should be applied to plant-microbe interactions on farms to generate more generalizable, predictive understanding of how (or, whether) changes in aboveground communities induced by farm management impact microbially mediated processes that are important for agriculture.

MICROBIAL FUNCTIONAL CAPACITY

Though microbial functional capacity is impacted by seasonal legume rotations (ch. 2), these changes are not significantly related to potential nutrient losses (ch. 3). This finding conflicts with research showing that experimental manipulations of microbial communities can be important drivers of these processes (Philippot et al. 2013). I hypothesize that this discrepancy is due to a relatively small (though significant) change in microbial diversity due to management and that these changes are not associated with changes in the particular genes responsible for carrying out these processes. Given significant interest in the connection between microbial diversity and ecosystem functioning (e.g. Torsvik and Øvreås 2002, van der Heijden and Wagg 2013), more research needs to focus on realistic, rather than experimental, losses of microbial functional capacity due to anthropogenic activity. More research is also needed that uses measures of microbial diversity that are more directly connected to ecosystem functioning. In this dissertation—and in much published work on microbial diversity—microbial communities are measured with DNA-based genomic techniques. Recent methodological advances have facilitated RNA-based techniques, which measure gene expression rather than presence and are therefore more directly related to microbial functional activity (Manefield et al. 2002, Whiteley et al. 2006, Bailly et al. 2007, Urich et al. 2008, Cardenas and Tiedje 2008, Blazewicz et al.

2013). Wider application of these techniques should lead to greater insight into factors driving microbial functional activity and its importance.

The impact of nutrient addition on microbial communities was different on experimental plots that had received fertilizers for two years than on actively managed farms that had applied fertilizers for at least seven years (ch. 2). On experimental plots nutrient addition was not associated with changes in microbial functional capacity, which was seen on active farms (ch. 2). I hypothesized that this may be due to a temporal decoupling in the response of taxonomic diversity and functional capacity, but more research is needed to confirm this pattern and identify the mechanism responsible. I hypothesized that one of the indicators of this temporal lag could be increased variability in microbial communities under high resource addition. Taxonomic diversity and community composition all had higher coefficients of variation on plots with greater addition of fertilizer (ch. 2). In theoretical and population ecology, increased variability of populations is an indicator of a shift to an alternate stable state (Scheffer and Carpenter 2003, Scheffer et al. 2009, Wang et al. 2012). Future research could shed light on whether increased variability in microbial communities can also indicate a shift to an alternate state under resource addition.

SOIL ORGANIC MATTER CHANGES AND EFFECTS

Soil organic matter is a key variable in soils (Palm et al. 2007) and its long-term build up is thought to be key for food security and C sequestration for climate change mitigation (Lal 2004, 2008, Luo et al. 2012). A growing paradigm of SOM suggests that the turnover of different SOM fractions are driven by separate mechanisms (Schmidt et al. 2011, Dungait et al. 2012). There is little understanding of how this new paradigm applies to predicting the relationship between SOM and ecosystem services. I show that stable SOM fractions are negatively related

with crop yields, while a more labile fraction is positively related to yields (under unfavorable weather conditions; ch. 4). More research is needed to determine how generalizable this pattern is. Broadly, most research on the benefits of soil C has focused on sequestration potential (McSherry and Ritche 2013, O'Rourke et al. 2015). More research is needed to generate predictive understanding of the relationship between changes in SOM stocks and the supply of ecosystem services from soils.

If SOM can be shown to be an important contributor to soil-based ecosystem services, indicators are needed to predict future changes in SOM stocks due to management. Though there is some evidence of rapid changes in SOM pools (Machmuller et al. 2015), most research suggests that SOM pools change over long time periods (Conant et al. 2011). This poses a challenge for management because management strategies need to be implemented over these long time periods to know if there will be an effect on soil C stocks. I suggest that changes in microbial functional capacity could be an indicator of longer-term changes in SOM (ch. 2). More research is needed to identify indicators of long-term changes in ecosystem processes that are important to the sustainability of agriculture.

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